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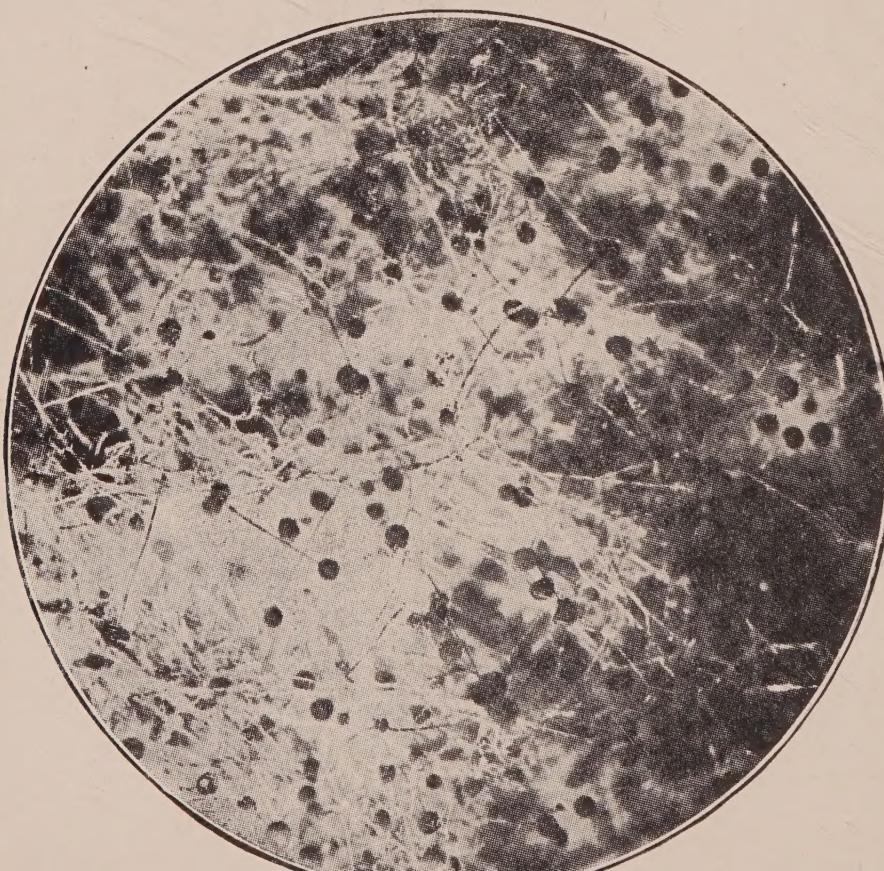
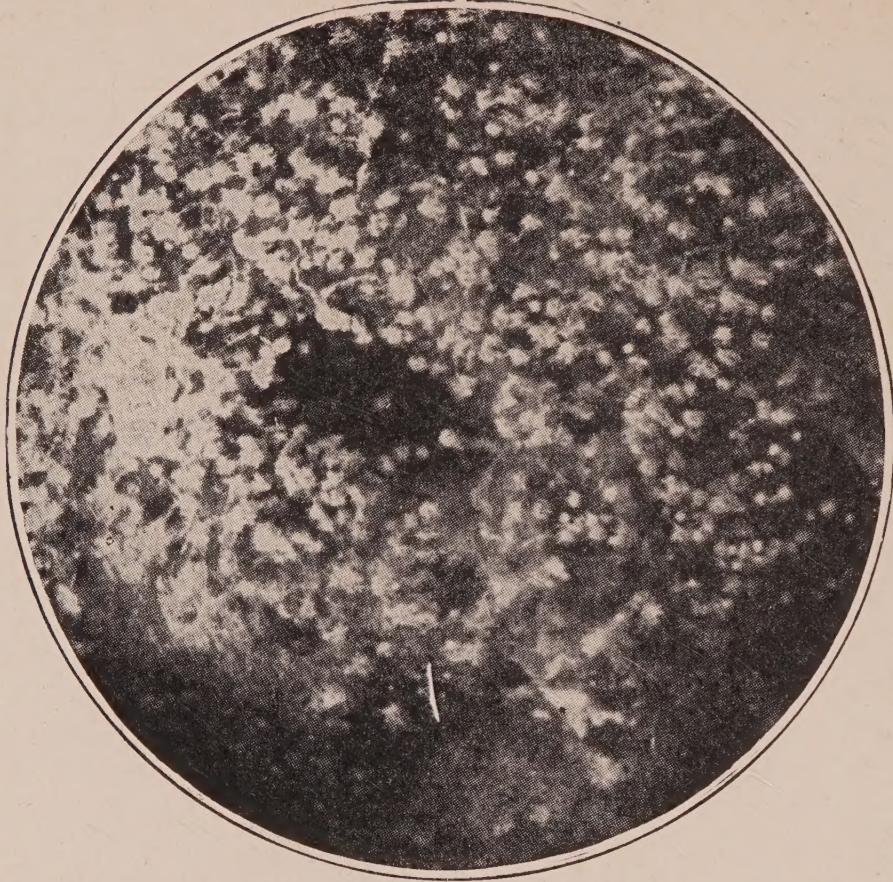
R.M. College.

No.

COMMON MOLD (*PENICILLIUM*), SHOWING MASSES OF SPORES

MOLD COLONY, SHOWING MYCELIUM AND SPORE CLUSTERS

[Both from micro-photographs made for this work]



87380

PRACTICAL DAIRY BACTERIOLOGY

PREPARED FOR THE USE OF STUDENTS
DAIRYMEN, AND ALL INTERESTED IN
THE PROBLEMS OF THE RELATION OF
MILK TO PUBLIC HEALTH : : : :

By

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ILLUSTRATED

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DEDICATION

To My Wife

*In appreciation of the constant assistance received
from her in this and other scientific works*

PREFACE

IT is about twenty years since the serious study of the bacteria in milk began; for while, in an incidental way, certain problems were investigated earlier, not until about 1890 was it recognized that the subject had a large field of practical applications. The development of Dairy Bacteriology has been due to the work of scientists on both sides of the Atlantic and this country has contributed no less than Europe to our present knowledge.

As the study has progressed it has appeared that the bacteriology of milk products has an intimate relation to two different classes of problems.

1. *The problems arising in the dairy proper* and affecting many questions relating to milk, butter and cheese. It is no exaggeration to say that the development of the knowledge and application of dairy bacteriology has revolutionized all phases of dairying, until all processes, from the milking to the final consumption of the completed product, have been modified by bacteriological knowledge. The time has come when it is no longer possible to be a successful dairyman without a knowledge of milk bacteria. To-day all courses planned for the study of dairying devote more or less time to the subject, and the student who fails to acquire some practical knowledge of the relation of bacteria to milk products is not properly trained to make a practical dairyman.

2. *The problems of the relation of milk bacteria to the public health.* The increasing proof of the distribution of disease germs by milk, the accumulating evidence that the health of the public, especially of children, is closely related to the bacteria in milk, and the growing realization of the conditions

of filth and unwholesomeness under which milk has commonly been produced, have led to the active interest, on the part of health officials, in the nature of the public milk supply and primarily in bacteriological problems. A lack of proper information on the part of health officials as to the conditions and possibilities of the dairy, and on the part of the dairymen as to the necessities and demands of the public and the method of meeting them, is to-day resulting in constant friction.

It is to meet the demand from both of these sources that the present book is written. It is designed on the one hand for dairy students and on the other for all who are interested in dairy products from the standpoint of their production, their distribution, or their consumption. For this reason it combines the practical with the theoretical.

In the first part is given a general discussion of the facts known concerning the relation of the bacteria in milk, and in the second part will be found practical directions for bacteriological analysis and methods of bacteriological study. It is hoped that thus the work may prove of use both as a manual for dairy students and as a reference book for dairymen and health officials who may be interested in all problems relative to the bacteria of milk.

H. W. CONN.

Middletown, Ct.,
October, 1907.

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PART I

GENERAL DISCUSSION OF DAIRY
BACTERIOLOGY

CHAPTER I

WHAT ARE BACTERIA?

BACTERIA were first seen two centuries ago by a Dutch microscopist, Leeuwenhoek, but were mostly forgotten for the century following. About 65 years ago it was first seriously suggested that these minute organisms might be of some significance in nature, and even at that time one unusual phenomenon of the dairy—blue milk—was attributed to the action of microscopic germs. About 1860 Pasteur showed a close connection between microscopic life and the souring of milk; but it was not until about 18 years ago that they were conceived to have a very intimate relation to dairying.

Dairy Bacteriology has practically all developed in the last two decades. The advance has been extremely rapid, and it is not an exaggeration to say that in this brief time bacteriology has revolutionized all dairy methods, from the cow to the consumer's table. The dairyman of to-day, and even more the dairyman of to-morrow, must understand bacteriology both theoretically and practically.

THE MICROSCOPIC APPEARANCE OF BACTERIA

The dairyman who is trying to do any practical work with bacteria in his dairy business does not ordinarily need to use a microscope. The organisms are so small that they must always be handled in masses, and for this purpose, though a microscope is extremely desirable, it is not absolutely necessary. The dairyman has rarely time to use a microscope properly. The student, however, who is learning of the bacteria in milk and their properties, must make a careful microscopic study.

Bacteria are all practically colorless, and as such are extremely difficult to see. It is almost impossible to study carefully a minute, colorless object under the microscope. For the study of bacteria, therefore, it is common to color them with staining materials, which render them more visible. The method of staining and study will be found on page 283.

Size.—Before we can enter into the study of their relations to dairying we must first learn what bacteria are and something also of their relations to conditions. The most striking fact about them is their extreme *minuteness*. (Fig. 1.) When we

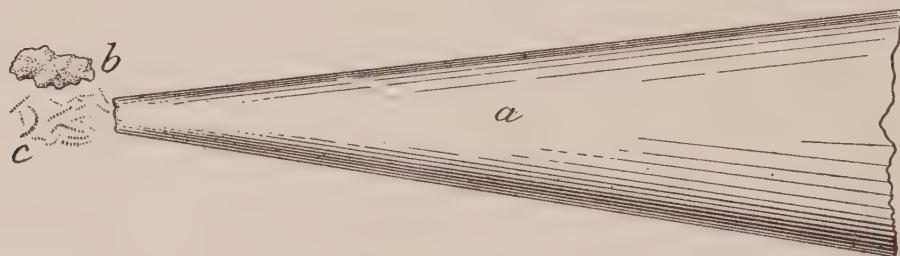


FIG. 1—COMPARATIVE SIZE OF POINT OF FINE NEEDLE (a), BIT OF DUST (b), BACTERIA (c)

hear of 100,000,000 in a single drop of milk we are apt to be incredulous, feeling that there could be no room for them, quite failing to appreciate their minuteness. There can easily be 100,000,000 in a drop and still be room for more. A space the size of a pin-head may hold 8,000,000,000, and 100,000,000 will have plenty of room in a drop of milk. This extreme minuteness must always be kept in mind in attempts to understand them.

This minuteness makes it impossible to handle them individually. The only way we can deal with them is to handle masses, and laboratory methods always begin with *cultivating* them, by which is meant, putting them into conditions where they will grow until large numbers may be obtained. Such masses or *cultures* we can easily deal with, and for this reason, in spite of the fact that they are the smallest known living

thing, most of the methods of bacteriological study do not even involve the use of a microscope.

The unit with which the student commonly starts is not a bacterium, but a *colony*. By reference to experiment No. 6 it will be seen that a colony is a mass of bacteria in some solid jelly medium. (Fig. 2.) Such a colony is composed of thousands of bacteria, all of which are supposed to have risen from

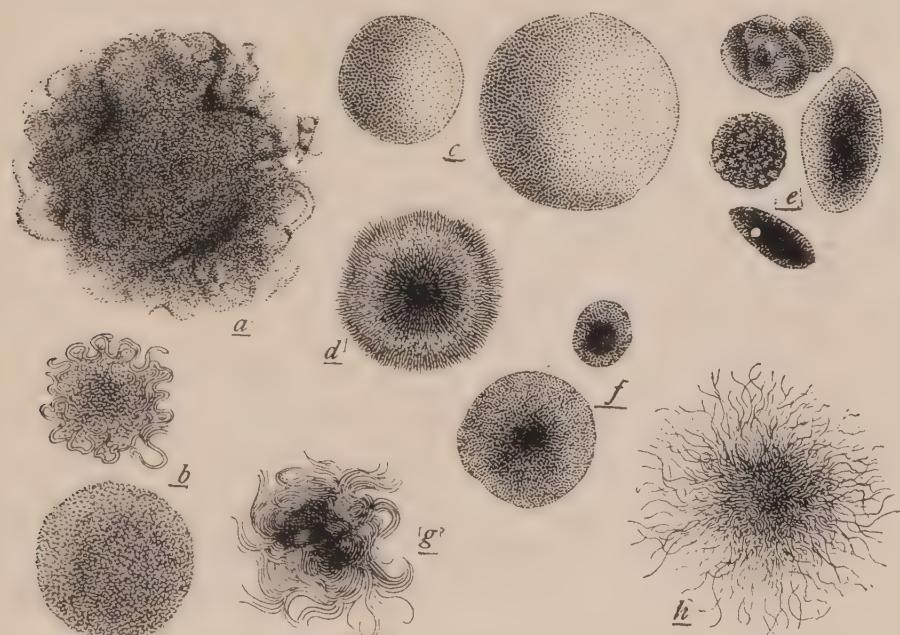


FIG. 2—VARIOUS TYPES OF COLONIES OF BACTERIA GROWING ON GELATIN

a single bacterium by multiplication. The whole makes a cluster big enough to be seen without any lens, and since all the bacteria in it have come from a single one they are all alike. The colony may be picked out of the jelly, placed in some medium in which the bacteria will thrive, and then, after proper growth, the bacteria can be studied by the microscope. Such cultures form the basis of bacteriological study.

Form of Bacteria.—Bacteria are of three quite different shapes, but all very simple. 1. Simple *spheres*. (See Fig. 3, *b*, *c*, *d*.) Such spherical forms are called *cocci*, and in

common microscopic preparation no internal structure can be seen, the bacteria appearing as faint stained balls. The cocci, however, differ somewhat in their method of growth, thus enabling the microscopist to distinguish different kinds, as will be mentioned presently. 2. The *rod formed* bacteria. (Fig. 3, c, f, g.) These organisms are longer than they are broad, some-

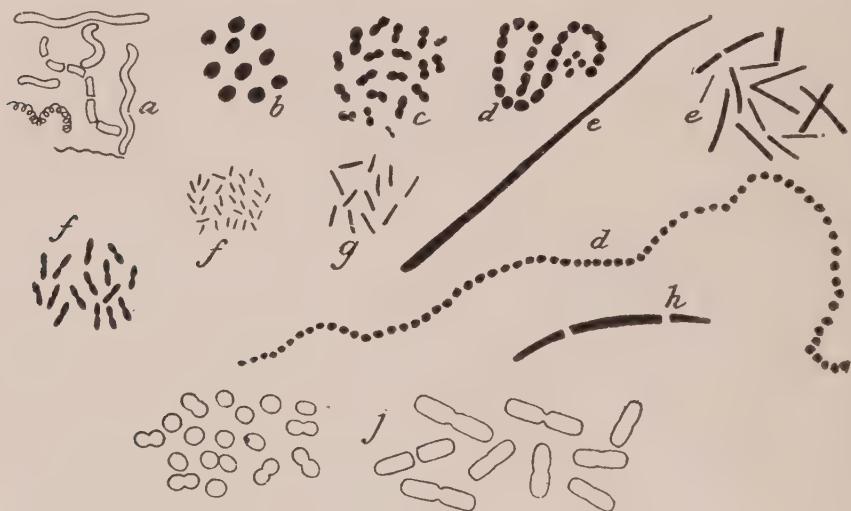


FIG. 3—SHAPES OF BACTERIA

a. Spirillum. b. Micrococcus. c. Micrococcus. d. Streptococcus
e—h. Rod-shaped bacteria. i and j. Divisions

times only slightly so, but at other times very much longer, forming, indeed, long, slender threads. 3. The *spiral formed* bacteria. These are either a long coil spiral or a very short one with only a single turn. (Fig. 3, a.) The type is rare in milk, and it is not likely that the dairyman will ever run across them, even if he makes extended microscopic studies.

Motility.—The next point of distinction among bacteria is based upon their motility. Some bacteria are capable of an active swimming motion, others are stationary. The motion is produced by minute, extremely delicate, vibrating hairs, called *flagella*. (Fig. 4.) The flagella are so delicate that they cannot ordinarily be seen in the living bacteria, and they do not stain by the ordinary method of staining. They are, there-

fore, never seen in the microscopic preparations usually prepared. They may be seen by special methods, but these are so difficult that the beginner cannot use them satisfactorily. The question of their motility is, however, usually determined without staining, by the study of the living bacteria. (Exper. No. 30.) These flagella are differently distributed upon different bacteria. Sometimes there is a single one on the end of a

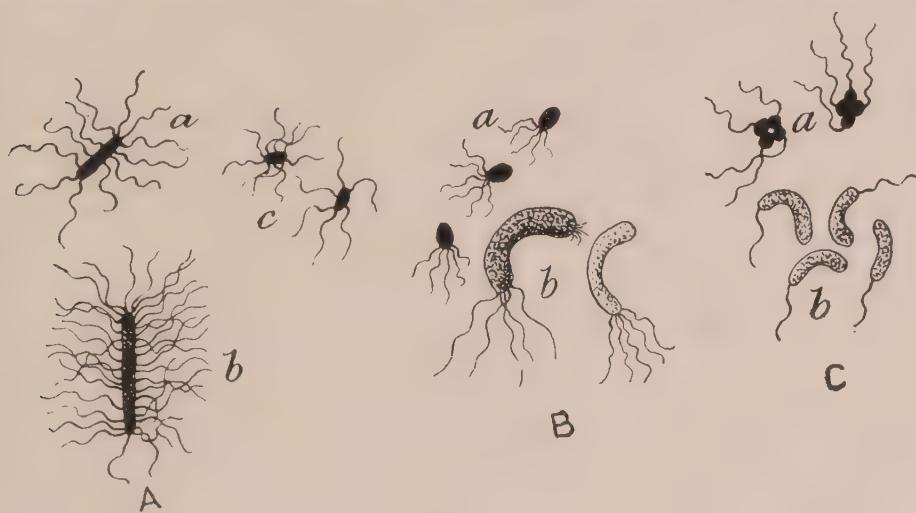


FIG. 4—FLAGELLA

A. Peritrichic. B. Lophotrichic. C. Monotrichic.

rod (Fig. 4, C)—*monotrichic*; sometimes a small tuft at one or both ends of a rod (Fig. 4, B)—*lophotrichic*; and sometimes there is a covering of flagella over the whole body of the bacterium (Fig. 4, A)—*peritrichic*.

Multiplication of Bacteria.—The most unique property of bacteria is their almost inconceivable rapidity of multiplication, and it is this which makes them of importance in nature. The method of multiplication of bacteria is the simplest conceivable. The organisms elongate a little and then break in two in the middle, two individuals thus arising where there was formerly one. Each of these now elongates and in turn breaks, and thus by continued division the numbers increase. Figure 3, *i* and *j*, shows this reproduction both in the rod and cocci forms of

bacteria. The rapidity with which these bacteria can grow and divide is surprising. In some cases it has been found that under favorable conditions of food and temperature, they may elongate and divide every half hour, and if they should continue to reproduce at this rate for twenty-four hours it is easily proved that a single bacterium would have by that time about 17,000,000 descendants. If each of these should continue to grow at the same rate, each would have, in twenty-four hours more, 17,000,000 descendants, and then the numbers would develop beyond a possibility of appreciation by human mind. It will, of course, be readily recognized that the bacteria do not long continue to multiply at this rate, for, if they did, in a few days there would be no room in the world for anything but bacteria. Their multiplication is constantly being checked by adverse conditions—lack of food, lack of moisture, etc.—and thus they do not on the whole very materially increase in numbers. But this inconceivable power of multiplication they do possess, and whenever they are placed for a few hours under conditions where they can have plenty of food and moisture for growth, they will develop with enormous rapidity, thus producing most profound changes in the substance upon which they are feeding. If they chance to develop in milk, they will cause a variety of changes which it is our purpose to study, for they change its physical and chemical nature profoundly. These changes, due to this enormous possibility of multiplication, have made bacteria of great importance in dairying and elsewhere in nature.

Production of Spores.—There is another method of producing new individuals, an understanding of which is of extreme importance in a knowledge of bacteria. It results in the production of new individuals, although it is hardly a multiplication since one individual only results from one to start with. The phenomenon in question is the production of *spores*, and it is illustrated in figure 5. The bacterium there figured consists of

rods. The contents of one of the rods collects itself in a spherical or oval body in the center of the rod. This later breaks out of the rod, the rest of the individual then dying and disappearing. The oval body itself is a *spore*, and is capable, when placed under proper conditions, of developing into a new rod. Inasmuch as only a single spore arises from a single bacterium, it is not a multiplication. Its purpose is not so much to increase the number of individuals as to enable the bacteria to endure adverse conditions without being killed. The ordinary bacteria are likely to be killed by being dried and will readily succumb to moderate heat, a temperature of 160° ¹ being sufficient to kill almost any of them. But these spores are covered with a hard case which enables them to resist the conditions which the active, growing and multiplying forms cannot resist. They may be dried for months, and even years, and still retain their vitality. They may be heated very much hotter than the active forms without injury; indeed, some of these spores may be boiled in boiling water for many minutes, for an hour even, or longer, without having their vitality destroyed, since, if the material is subsequently cooled, the spores are capable of generating and growing into new bacteria. As the result of this it will follow that, while it is very easy to kill ordinary bacteria by heat, it is far more difficult to destroy spores. Some species of bacteria produce such spores while others do not, and hence some are much more easily killed by heat than others. Milk, for example, contains many kinds of bacteria. By the simple boiling or,

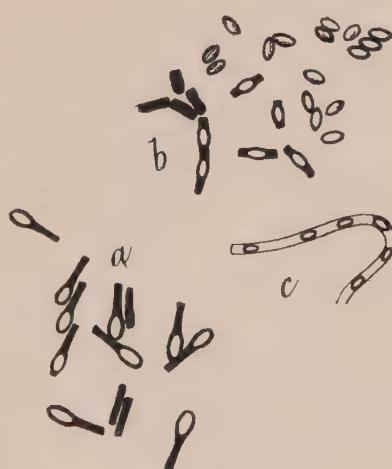


FIG. 5—SPORE PRODUCTION

¹ Temperatures used in this book always refer to the Fahrenheit scale.

indeed, the heating of the milk to a temperature of 160°, a vast majority of the bacteria are killed; but the few spores that may chance to be in the milk are not thus killed, and these will be able subsequently to develop. If milk contains spore bearing bacteria, it cannot be sterilized by boiling, and since it almost always does contain them, boiling is not sufficient to sterilize milk. This phenomenon of the high-resisting powers of spores must always be borne in mind in all problems of sterilizing.

YEASTS

Among the micro-organisms that one is likely to find in milk

are some that do not belong to the bacteria proper, although closely related to them and of hardly less importance in dairy phenomena. The first type is YEAST. Yeasts are microscopic cells of a round or oval shape, and may usually be distinguished from any of the true bacteria by their somewhat larger size. The relative size of yeast cells and bacteria cells may be seen from figure

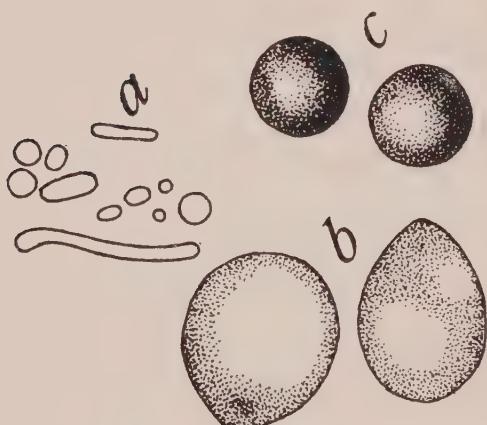


FIG. 6—RELATIVE SIZE OF YEAST (b)
MOLD SPORES (c) AND BACTERIA (a)

6. Occasionally we find small yeast cells and sometimes large bacteria, so that the matter of size is not in all cases sufficient to enable one to determine whether he is dealing with bacteria or yeasts. The primary difference between the two is in their method of multiplication.

The multiplication of yeasts is different from that of bacteria, and is known as the multiplication by *budding*. There appears on the side of the yeast cell a minute bud which continues to increase in size (Figs. 7 and 8), until after a time it becomes as large as the cell from which it was produced. The

two may break apart at once, or each of them may in turn produce other buds before separating; but in either case there are now two cells where there was originally but one. Yeasts also multiply with considerable rapidity, but not, as a rule, as rapidly as many of the bacteria; nevertheless their great power of multiplication makes them of great importance among the forces of nature. The reproduction by budding is the primary characteristic which separates yeasts from bacteria, and the

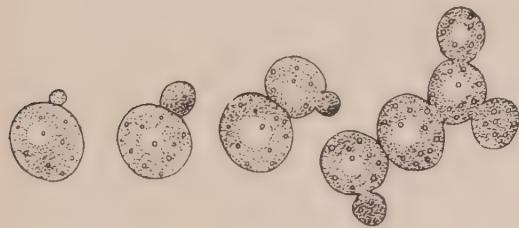


FIG. 7—COMMON YEAST
Showing multiplication by budding

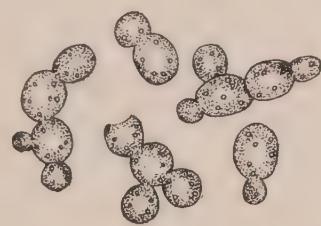


FIG. 8—YEAST IN FERMENTING CIDER

Sometimes called "wild yeast," coming directly from the air

determination of the method of reproduction will always enable one to tell whether he has a yeast or a bacterium under study. When, however, one finds under his microscope rather large, round or oval cells, he is pretty safe in concluding that they are yeasts rather than bacteria, and if he can find small cells attached to the larger ones—buds—he may be absolutely sure that he is dealing with yeasts.

A second distinction between yeasts and bacteria is in the type of fermentation they produce. Bacteria produce *decay*, *putrefaction* and *lactic acid* fermentation. Yeasts are more commonly associated with the *alcoholic* fermentation, by which some forms of sugar are easily converted into alcohol and carbon dioxide. The latter type of fermentation does not usually occur in milk.

The yeasts do not play quite so important a part in dairying as bacteria, and yet, as we shall see later, in some phases of the subject they are of great importance.

MOLDS

These are plants of considerably larger size and much higher structure, and they do not logically belong to the consideration of bacteria in milk.

Their relations to milk and milk products, however, are in many respects very much like those of bacteria, and in certain phenomena associated with the ripening of cheeses they are of as great importance as bacteria themselves. It is necessary, therefore, for the dairyman to know something about them. They are plants that in all cases are large enough to be seen with the naked eye, although the microscope is required to study them. They consist of a mass of branching threads of very minute size. (Figs. 9, 10 and 11.) Sometimes the threads are large enough to be seen without a microscope and sometimes not. This mass of threads is almost always colorless, or of a pale white, and grows on the surface or in the substance of various materials upon which the plant feeds.

On cheeses, for instance, it thrives in profusion upon the surface, and sometimes extends into the cheese itself.

There are a great many species of molds, but they are all very much alike so far as concerns the formation of this colorless thread (*mycelium*), although there are some differences to be seen here. But molds have methods of multiplication which commonly enable us to distinguish different species. After they have grown luxuriantly, they usually send minute branches into the air, the ends of which finally produce rounded spores, or reproductive bodies. These are sometimes produced in chains, sometimes in masses, and the method of

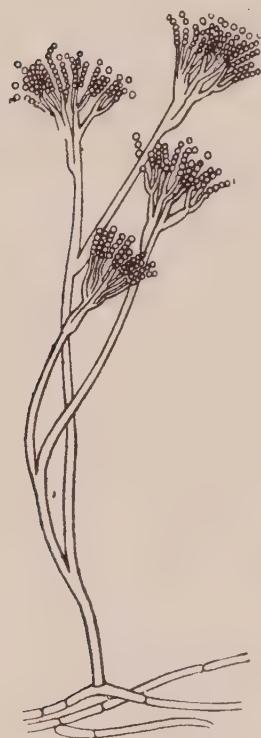


FIG. 9—COMMON MOLD,
PENICILLIUM

Method of forming spores

spore formation is the chief means by which the microscopist separates the different figures from each other. Figures 9 and 10 show the method by which some of the common molds produce their spores. The studying of molds for the purpose of dis-

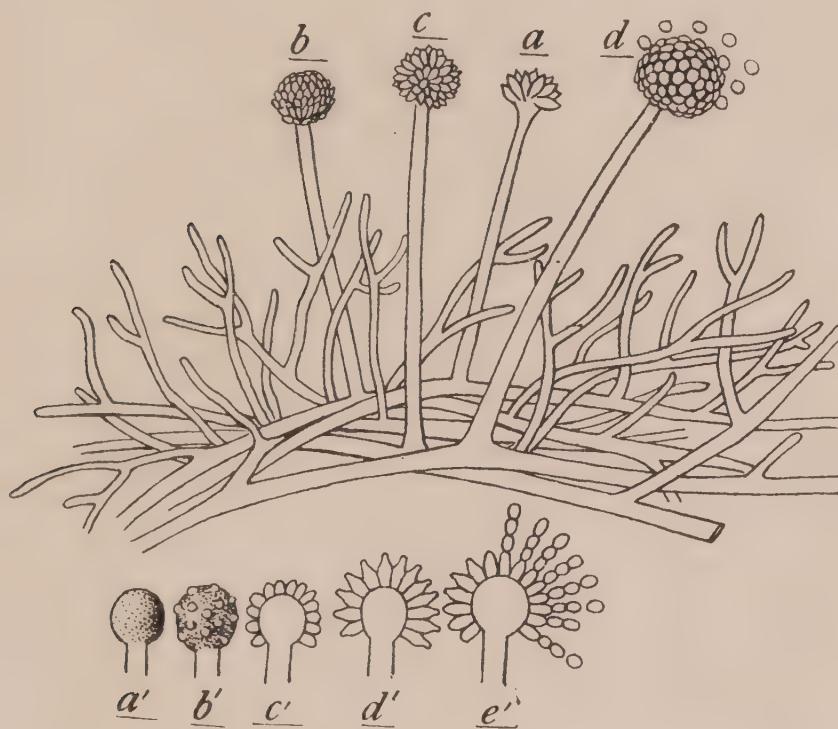


FIG. 10—COMMON MOLD, *ASPERGILLUS*
Mycelium and method of forming spores

tinguishing spores does not, however, belong to our immediate subject. Such types as are concerned in dairy problems will be mentioned later in the proper places. (See frontispiece.)

NAMES APPLIED TO BACTERIA

The detailed classification of bacteria is rather complicated and difficult, and need not detain the dairy student. A few terms, however, are so general that it is necessary to learn their meaning, especially since there is considerable confusion in

their use. The following brief classification will explain the meaning of the common terms in use:

Large colorless plants forming

DELICATE THREADS and frequently

colored spores.....**MOLDS**¹



FIG. II—MOLD SHOWING THREADS OF THE MYCELIUM (MAGNIFIED)

Single celled plants, multiplying

BY BUDDING. (Fig. 7).....**YEASTS**

Single celled plants, multiplying

BY DIVISION. (Fig. 3).....**BACTERIA**

¹ Other fungi, like mushrooms, would belong here, but play no part in dairying, and may here be neglected.

BACTERIA

SPHERICAL BACTERIA:

Dividing in *one plain* so as to form
chains. (Fig. 3, *d*) **Streptococcus**

Dividing in *two plains* and not
forming chains. (Fig. 3, *b, c.*) **Micrococcus**

Dividing in *three plains* and
forming cubical masses. (Fig. 13.) **Sarcina**



FIG. 12—MOLD COLONY ON A GELATIN PLATE

ROD SHAPED BACTERIA:

With flagella and consequently
motile. (Fig. 4, *a, b.*) **Bacillus**

Without flagella and consequently
non-motile. (Fig. 3, *e, f, g.*) **Bacterium**

SPIRAL BACTERIA. (Fig. 3, *a*).....**Spirillum**

The genus **BACILLUS** is further divided as follows:

Bacilli with only *one* flagellum (Fig. 4, *C*)

are named.....**Monotrichic Bacilli, or Pseudomonas**

Bacilli with a *tuft* of flagella at one end

(Fig. 4, *B*) are called.....**Lophotrichic Bacilli**

Bacilli with flagella *over the whole body*

(Fig. 4, *A*) are called.....**Peritrichic Bacilli**

It will thus be seen that the term *Bacteria* applies to the

whole group of organisms that multiply by division, whose study constitutes the study of Bacteriology, while the term *Bacterium* refers to a

single division of the group, viz: the non-motile, rod forms. The term *Bacillus* should apply to

motile forms only. The names *Bacillus* and *Bacterium* are sometimes confused; for example, the

tubercle *Bacillus* is really a *Bacterium* since it is

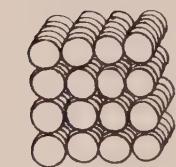


FIG. 13—A
SARCINA TYPE

non-motile, but the name *Bacillus* was given it years ago before the above distinctions were recognized, and it has not been changed. Some other bacteria named twenty years ago retain their earlier names in some books, but they are slowly having their names brought into harmony with the above distinctions.

The term *Coccus* is applied to any spherical organism of the group bacteria.

PLANTS OR ANIMALS

The question whether bacteria are plants or animals has been much discussed and answered in both ways. The reason for the uncertainty is the fact that our biologists are unable to give any clear distinction separating animals from plants, and with any definition that they can give, bacteria show relations to both plants and animals. The method of forming spores allies them to plants, while their frequent motility, together with the fact that they live upon foods similar to animals, allies

them to the animal kingdom. After long discussion it has been quite definitely concluded that they are closely allied to yeast, and these are in turn closely related to molds. Biologists agree that molds are plants, and, therefore, the conclusion to-day is that bacteria are also plants, but of an extremely low type. It is a matter of no special significance, excepting to the scientist, whether we consider them as belonging to one or the other. Their relations to the dairyman are the same whatever we call them.

RELATIONS TO CONDITIONS

Temperature.—The bacteria behave very differently at different temperatures. Their rapidity of multiplication is very decidedly modified by the different degrees of temperatures. In general, at low temperatures, multiplication is comparatively slow. At freezing they do not multiply at all; but when the temperature is raised above freezing they begin to grow, and their rate of multiplication increases with the increase in the temperature up to a certain point, an *optimum temperature*. If the temperature is raised higher, the rate becomes slower again, until finally they stop growing. If the temperature is raised still higher, they are injured; and finally by a certain amount of heat they are killed. Thus there is: 1. A *minimum temperature*, the lowest at which they will all grow. 2. An *optimum temperature*, at which they will grow best. 3. A *death temperature*, which will kill them.

Minimum Temperature.—Some species will grow, though slowly, at temperatures only just above freezing, multiplying at 33° , while other species will not grow at all at such temperatures. Some will hardly grow at temperatures lower than that of body heat, 98° . Others again seem to require a temperature much higher than this, even as high as 120° to 130° . Between these two limits different species show all kinds of variations.

Optimum Temperature.—This also differs very much for different species of bacteria. Some will grow at 60° to 65° ,

others at 130° . But the majority of them grow most rapidly at temperatures somewhere between 80° and 100° , a fact that explains the rapidity of the souring of milk in hot weather, and the quickness with which any organic material decays during the summer. Above 100° most bacteria are injured, and if the temperature is raised, they soon stop growing. There are, however, a few species which grow at unexpectedly high temperatures; some types are found that grow most rapidly at a temperature as high as 140° and some even higher. These peculiar bacteria are called *thermophiles*. Such bacteria may be found in milk by proper means, but it is needless to say that they have no important relations in dairy problems, for dairy products are never kept at a temperature as high as these.

Death Temperature.—Most active bacteria are killed by a temperature of 150° kept up for half an hour. This temperature may, therefore, be satisfactorily adopted for pasteurizing. (See page 151.) At this temperature, however, they die slowly, while a temperature of 160° destroys them much more quickly, and 170° to 180° more rapidly still. Hence, the higher temperatures are usually adopted for pasteurization. A total destruction of bacteria, including their spores, can be brought about only by temperatures above that of boiling water, and this is usually accomplished in the case of liquids in a closed chamber, in which the steam can be generated under considerable pressure. If the steam is allowed to collect in such a chamber at a pressure of 115 lbs., the temperature, then, will be about 240° . This temperature kept up for half or three quarters of an hour destroys even the most resisting spores and may be relied upon to produce the perfect sterilizing of any liquid. Laboratories usually have a small apparatus designed for this purpose, called an *autoclav* (Fig. 76), and this is used constantly for sterilizing of liquids.

Relation to Cold.—While heat will destroy all bacteria, cold will not do so. It is practically impossible to destroy the life

of bacteria by freezing, at least with any certainty, for no matter how low the degree of temperature used, the life of some of these organisms seems to be totally resistant to cold. Experiments have shown that bacteria cooled to the temperature of liquid air, or even liquid hydrogen, are not killed, and after being warmed again still retain their life. Although these extremes of temperature do not destroy all bacteria, the simple matter of freezing and thawing will kill a great number of individuals. If water containing a large number of bacteria is frozen and subsequently thawed, the bacteria will be found much reduced in numbers, although they are not by any means all killed. When, therefore, water is contaminated by sewage containing typhoid bacteria, and ice is collected from it for domestic purposes, the typhoid bacteria may still be left alive in the ice. Such ice may still be a source of danger. But it must also be remembered that the freezing destroys a very large proportion of these germs, so that the danger from this use of ice is far less than the use of water from the same source.

Relation to Air.—Nearly all living organisms require air, and it was formerly supposed that nothing could live without it. Certain types of bacteria, however, are able to live without air. Indeed, some species, while they grow readily if they have no contact with air, fail to grow at all when the slightest amount of air is present, growing only in the absence of oxygen. This type of bacteria is spoken of as *anaerobic*. At the other extreme there is a long list of bacteria which can grow only in the presence of air, failing to grow if they do not have oxygen at their command. This is the type of *aerobic* bacteria. Between the two is an intermediate group capable of growing either in the air or out of contact with it, and this type is spoken of as *facultative anaerobic*. The most important of all dairy bacteria belongs to this class, the common lactic acid bacterium, *Bact. lactis acidi*.

Relation to Moisture.—Bacteria will grow only in the presence of considerable quantities of moisture; indeed, they demand more moisture than most organisms. They will hardly grow at all unless there is 30 per cent. of moisture in the material in which they are living, and even then the growth is slow. On the other hand, they flourish most luxuriantly in localities where the water is from 90 to 100 per cent. Hence, as materials dry, bacteria will cease to grow in them, and any substance that can be dried can be thoroughly protected from their action. This explains why dried fish and dried meat, fruits, dried milk, etc., will keep indefinitely. The drying, however, does not actually kill the bacteria, for although they do not grow when the water is extracted from them, they may remain alive for weeks, months, or even years. In other words, it is impossible to depend upon drying as a means of destroying bacteria, for while many individuals will fail to live, many others do not seem to be injured at all by the drying, and are capable of resuming life again as soon as they find moisture.

Relation to Food.—Bacteria are just as dependent upon food as are larger organisms, and they feed upon a greater variety of materials, perhaps, than any other organisms. There are some that appear to be capable of feeding upon what is called *inorganic* food; that is, upon mineral materials. The vast majority of bacteria feed upon *organic* material, by which is meant substances that have been produced by other animals or plants. Their relation to food is very much like that of the animal kingdom, and in general they feed upon the same kinds of food that animals do: *meats, gluten, milk, eggs, etc.* It is of importance to note, however, that whereas they feed with great readiness upon *proteid* foods, like the lean part of meat, white of eggs, gluten of wheat, legumen of beans, peas, etc., they do not feed, as a rule, upon sugar or starches. *Yeasts*, on the other hand, are adapted to feed upon sugars. We find, there-

fore, that of the three great food products upon which animals feed, *proteids*, *carbo-hydrates* and *fats*, the bacteria feed especially upon the proteid foods, and but rarely upon the carbohydrates. There is one conception of importance to the dairyman. Certain types of bacteria act upon milk sugar (a carbo-hydrate), converting it into lactic acid and causing the milk to sour. There are some kinds that are capable of acting on fats.

The food which bacteria consume may be either living or dead when they attack it. In the case of most bacteria the organisms are unable to feed upon the material while it is alive. If bacteria, for example, are placed upon living muscle, they are unable to attack it and soon die; but if they are placed upon the same muscle after it is dead, they feed upon it readily and cause it to putrefy. These organisms which feed upon lifeless bodies include the vast majority of bacteria. There are, however, other species of bacteria that are capable of living upon the bodies of animals and plants while these are still alive. Inasmuch as they can feed upon living organisms they are liable to produce disease and constitute in general the disease bacteria. Bacteria feeding upon living animals and plants are called PARASITES. Bacteria feeding upon dead animals and plants are called SAPROPHYTES. Both saprophytes and parasites are of great importance in dairy problems.

ENZYMES OR CHEMICAL FERMENTS

In order to understand some of the topics discussed in the following pages it is necessary to recognize the distinction between *organized* and *unorganized ferments*. The former term applies to bacteria and yeasts which produce their action by **growth**. They are actual living bodies which grow and multiply. When they produce a fermentation, like the alcoholic or the lactic acid fermentation, they increase in numbers during the process; that is, they act by *growth*. The unorganized fer-

ments, *enzymes* or *chemical ferments*, are not alive. They appear to be chemical substances which produce their fermentations by their simple presence. They do not live, grow or multiply, but they have remarkable powers of bringing about changes in other bodies. While these enzymes are not alive, they are always produced by living bodies and from no other source. For example, the living cells in the glands in the stomach produce one of these enzymes, which we call *pepsin*, and which has the power of greatly changing the chemical nature of the swallowed food while it is in the stomach. Frequently similar enzymes are secreted by bacteria.

In the study of bacteria we are, of course, dealing primarily with the living or organized ferments. But in some cases, as we shall see, they produce their action directly through the secreted enzymes, and thus in their study we have to consider both classes of action. It is always important to bear in mind that the organized ferments are ever increasing in numbers, when they have proper food, while the enzymes do not thus increase in amount, except as they are secreted by living cells. If we stop the life of bacteria by an antiseptic, we stop its fermentation, but such antiseptics will not stop the action of an enzyme, since an enzyme acts chemically and not by its life.

CHAPTER II

TYPES OF BACTERIA FOUND IN MILK

IN the study of the types of bacteria that may be found in milk, three different factors are to be considered: 1. Their action on milk, and its relation to all who deal with this product. 2. Their relation to butter and cheese making. 3. Their relation to the human organism: and, therefore, their effect upon the persons using the milk as food. Certain bacteria that are troublesome to the dairyman are perfectly wholesome to the consumer, while, on the other hand, some bacteria that are very injurious and even fatal to the consumer are of no special significance in dairying, so far as their effect upon milk is concerned. We shall also find that organisms which are of great nuisance to a person dealing in milk are favorable and, indeed, indispensable to butter and cheese making. These problems must, therefore, be considered independently.

It will be most convenient to study milk bacteria under the heads of normal and abnormal types. Since milk does not usually contain any bacteria at the time when it is secreted from the milk glands, there are really no such things as normal bacteria. Nevertheless, it is practically impossible to procure milk from any cow, no matter under how cleanly conditions it may be kept or how carefully we work, in such a manner that it will be sterile by the time it reaches the milk pail. We may, therefore, recognize bacteria as inevitable, and the distinction between normal and abnormal bacteria will, therefore, be as follows: *Normal bacteria* are those that under all ordinary circumstances are practically certain to be found in milk; their presence must always be expected, and it is practically impossible to avoid them. *Abnormal bacteria* are those which are

less common, occurring in milk under certain conditions but which can generally, by proper means, be prevented from getting into the milk in quantities large enough to produce any effect.

NORMAL MILK BACTERIA

The Souring of Milk.—The most common change which occurs in milk is its souring. This has always been recognized and it has been supposed to be a natural change in the milk, the supposition being that milk would sour, just as blood will clot. The studies of bacteriologists have shown, however, that this is a mistake. The souring of milk is not a characteristic of the milk itself, for it will not occur in perfectly pure milk. It is always brought about by the growth of bacteria. Nevertheless, it is of almost universal occurrence even in good milk. Indeed, if one obtains a lot of milk which will not sour when kept for a day or longer in a warm temperature, it at once arouses suspicion that some means have been adopted for keeping the milk sweet, either by the use of preservatives, or by pasteurization. This is not necessarily the case, however, for, under some conditions, pure milk may be obtained which will not undergo the ordinary souring; but the phenomenon is so common that it is to be expected in all samples of normal milk. The souring is the result of the production of lactic acid from milk sugar. Milk contains about 4 per cent. milk sugar and, under the action of certain bacteria that grow in milk, this milk sugar is changed into lactic acid. The change is frequently expressed by the following formula:



There is no question, however, that this does not represent the change that actually takes place.

A molecule of milk sugar contains a certain multiple of the formula above given, but the multiple is not known, and the change into lactic acid is a far more complicated one than

would be indicated by the simple equation above. Its exact nature is not understood even by chemists, and it is sufficient for the dairyman to know that milk sugar is changed to lactic acid. The lactic acid causes the milk, first, to turn sour, as indicated by chemical test, or by its taste; and, later, when the acid rises to .7 or .9 per cent., the milk curdles. This phenomenon is also a somewhat complicated one. Milk contains casein in a state of partial solution. As long as the liquid is slightly alkaline, this partial solution is maintained, but the casein will not remain dissolved in an acid solution. As soon as the liquid becomes sufficiently acid, the casein, by a well known chemical law, is precipitated and the milk curdles. Any other acid will produce exactly the same result, as may be proved easily by pouring into milk a little vinegar, or any other acid. The curdling of milk is produced by the appearance of an acid reaction. Curiously enough the three factors, bacteria growth, milk sugar destruction and acid formation, though causally connected, do not run parallel, sometimes one and sometimes another of the three running ahead of the others. In other words, it is impossible to determine the amount of acid from the number of bacteria present.¹ The reasons for this are not well understood.

Souring of Milk and Thunder Storms.²—We have just noticed that the souring of milk is always the result of bacterial action. There has been a wide-spread belief that thunder storms bring about the souring and curdling of milk, and the general opinion has been that this is due in some way to the direct action of thunder or lightning. It frequently does happen that milk will sour and curdle during a thunder storm, but the conclusion that there is any real connection between the two is a mistake. The suggestion that electricity has some direct effect upon milk was advanced some years ago and carefully tested;

¹ Haacke. *Arch. f. Hyg.* xliv., p. 16, 1902.

² Treadwell, *Science*, xvii., p. 178, 1894.

but the result demonstrated beyond a doubt that the electric current will not sour milk. It was then suggested that the electricity of the thunder storm produced ozone in the air, and this hastened the souring. This ozone theory, also, was carefully tested and proved to be without foundation, for ozone in itself has no power of affecting milk. Moreover, further experiments proved that, unless bacteria are in the milk, thunder storms have no effect. Milk that is deprived of bacteria may be kept for months during the whole season of thunder storms and never show the slightest trace of souring, showing, of course, that thunder storms have no direct action upon the milk, since they never affect milk from which the bacteria have been removed. Practical experience has also shown that if the milk is kept sufficiently cold in an ice chest, or by other means, the thunder storms have no effect upon it. Only milk that is not sufficiently cooled, or that is not cooled at all, is affected by thunder storms. From all these facts it becomes evident that if thunder storms have any effect upon milk it is only indirect.

The reason that the souring of milk frequently occurs at the same time with thunder storms seems to be very simple. Bacteria will grow most readily at warm temperatures. Thunder storms occur at seasons of the year when the temperature is high; and the same atmospheric conditions which bring on thunder storms are likely, and, indeed, certain, to stimulate the growth of bacteria. If the temperature is high, and the milk is not sufficiently cool, it will sour very rapidly, whether any thunder storms are occurring or not. Hence it is not strange that, after a thunder storm, we often find milk which has not been kept cool, soured and curdled, and our natural inference is that the thunder storm has had some connection with it. The whole phenomenon appears to be one of bacteria growth, stimulated by warm temperature. The souring of milk by thunder storms may be absolutely prevented by keeping the milk cool.

Varieties of Bacteria Which Sour Milk.—The bacteria which sour milk are known, in general, as *lactic acid bacteria*. There are many varieties of them, however, differing more or less from each other. A very large number of different acid bacteria have been separated from milk by bacteriologists, and have been described. Exactly how many of them there are it is impossible to say. The reason for this impossibility is the fact that up to the present time we do not know what constitutes a type or a species of bacteria. We know that large numbers of lactic bacteria have been found which differ from each other in certain small points. Whether these differences are enough to separate them into different types or species, or whether they are only slight variations which appear in one and the same organism, is not yet known. We do not know, moreover, to what extent these slight differences may come and go under different conditions. For example, the various types of this class of organisms show great variation in the rapidity with which they can curdle milk. Some of them curdle it in a very few hours where the temperature is warm, others require several days for producing the same effect, while still others will not curdle the milk under any circumstances. Whether these should be recognized as different varieties, or simply as modified forms of the same type has been a subject of dispute. It is certain that this power of curdling milk may be increased in the same organisms by proper treatment in the laboratory, and the general opinion, to-day, is that some bacteria which curdle milk and others which sour it without curdling, cannot be regarded as separate species, but only as modifications of the same. Similarly in regard to other slight differences, our bacteriologists are not certain as to how wide variations may be found in the same organism. For this reason it is impossible to state how many species of lactic acid bacteria there are. Although this is true, it has become evident in the last few years that the lactic acid bacteria separate themselves into two some-

what distinct groups, more or less sharply separated from each other. The more bacteria are studied, the more clear it becomes that these groups represent very distinct types, each one of which may have a large number of varieties differing in slight points. We shall, therefore, understand the lactic bacteria best if we briefly consider the characteristics of these primary types.

BACTERIUM LACTIS ACIDI—(*Streptococcus lactarius*)

This organism is the lactic acid bacterium of milk, *par excellence*, being the type that, in the vast majority of cases, produces the souring and curdling of market milk. It is common, not only in milk from all sections of the United States, but also in all the markets of Europe, and must, without doubt, be looked upon as a common cause of the souring of milk. It is interesting to note that this species was not the first lactic acid bacterium found in milk, and described as the cause of milk souring. One of the other types mentioned below was found earlier, because it produces a large colony on gelatin. But when milk is studied by our more modern methods, this *Bact. lactis acidi* type is found to be by far the most common.¹ It is not to be inferred from this statement, however, that it is always the cause of sour milk, for, while it is the cause in the majority of cases, not infrequently samples of milk are found that have been soured by one of the other types mentioned later.

It will be noticed that two names have been given above to this organism. These are not, by any means, the only names that have been applied to it, for it has been found by many bacteriologists, many of whom have given it distinct names, not knowing that they were studying an organism already named. The reason why we have given the above two names

¹ Esten. Ann. Rep. Storrs. Exper. Sta., p. 44, 1896.

Kozai. Zeit. f. Hyg., xxxviii., p. 386, 1901.

Weigmann. Cent. f. Bact. II., v., p. 825, 1899.

Günther and Thierfelder. Arch. f. Hyg. xxv., p. 164, 1895.

should, however, be explained. The organism in question appears commonly in the form of a short, non-motile rod, which is not infrequently divided into two, as shown in figure 15, *c*, giving the form known as the diplococcus. When it appears in this form, the question arises whether it is not more properly called one of the coccus type than a Bacterium. The longer forms, as shown in figures 14 and 15, may simply represent the cocci, elongating and about ready to divide. If this is the case, the organism should not be called a Bacterium, which term is, as we have seen, applied to rods, but rather *Streptococcus*, which is the name given to spherical forms having one division plane. (See p. 13.) If it is a coccus form it should be called *Streptococcus*; then the name *Streptococci lactarius* is the correct one to adopt.¹ If, however, the organism is regarded as a short rod,

then the term Bacterium should be applied, and the name *Bact. lactis acidi* should be used.² It may seem strange that bacteriologists cannot agree as to whether this most common milk organism is a Bacterium or a *Streptococcus*, but the fact is, that there is no sharp criterion by which the microscopist can tell whether he is dealing with an extremely short rod or a spherical form that is slightly elongated just before division. The same culture of bacteria from which figure 14, *d*, was drawn was submitted to two microscopists, one of whom called it a Coccus and the other a Bacterium. Because of this difficulty in finding any sharp distinction between the two, it has been impossible to state with absolute certainty

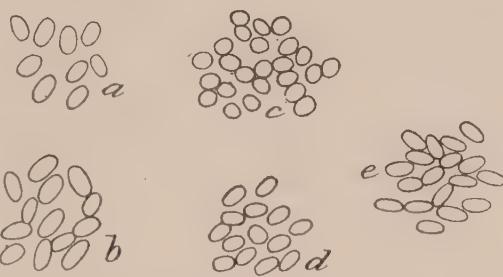


FIG. 14—VARIETIES OF BACT. LACTIS ACIDI (CONN)

¹ Kruse. Cent. f. Bact. II., xxxiv., p. 737, 1903.

² Leichmann. Cent. f. Bact., II., pp. 281 and 777, 1896; V., p. 344, 1899.

whether this organism is properly called a Bacterium or a Streptococcus. The term Bacterium, however, has been applied to it for nearly twenty years, and only in the last year or

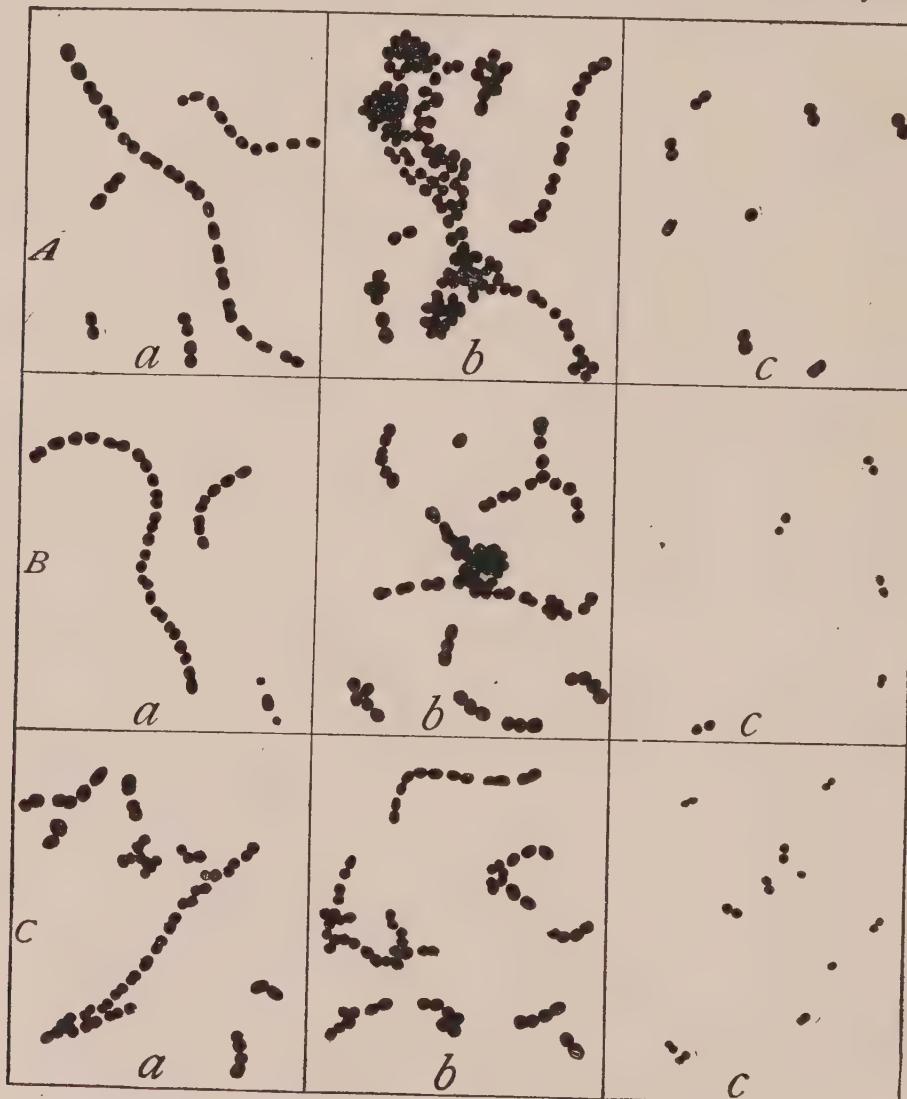


FIG. 15—VARIETIES OF BACT. LACTIS ACIDI (HEINEMANN)

two have some suggested that it ought to receive the name of Streptococcus. This recent suggestion has not as yet been very favorably received, and it is still known, and for some time, doubtless will be known, in literature, by the name of *Bact. lactis acidi*, although the name Streptococcus is taking its place.¹

¹ Heinemann. Jour. of Infectious Diseases, III., p. 173, 1906. Jour. of Infectious Diseases, IV., p. 1, 1907.

It is possible that this type should really be divided into two, one of which is a *Streptococcus* and the other a *Bacterium*. This seems to be the conclusion of recent works.¹ But these two are in other respects almost alike and in our review here it is not wise to try to make this distinction.²

Isolation of *Bacterium Lactic Acidi*.—It is an extremely easy matter to obtain this organism from milk, as follows:

A sample of milk that has become somewhat sour, but has not yet curdled, should be used for the purpose. This will contain millions of bacteria, and should be very highly diluted for the experiment. A dilution of one to a million is not too high for the purpose. Litmus gelatin plates should be made as described in experiment II. These are to be kept at about 70° for two to four days when they will be found filled with minute red points, or colonies. These are colonies of lactic acid bacteria. On such a plate will likely be found, however, several kinds of colonies, and perhaps two or three types of acid colonies. But the colonies of the *Bact. lactis acidi* can, when the plates are properly made, be distinguished from others by the use of a low magnifying power under the microscope. Each of the colonies is found to be quite red, very small, and growing wholly underneath the surface of the gelatin. Examination with a microscope shows an appearance like figure 16, the most

¹ Löhnis. Cent. f. Bact., II., xviii., p. 97, 1907.

² Besides the names given above the following names have been applied to lactic acid bacteria that are in essential respects like the one described and should at least be regarded as belonging to the same group:

Bact. or Streptococcus Güntheri.

Streptococcus acidi lactici.

Bact. acidi paralactica.

Bact. lactis acidi aromaticum.

Bact. lactis acidi maltigeum.

Bact. lactis acidi purum.

Bact. lactis acidi acerbum.

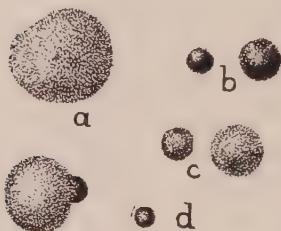
Staphylococcus lactis acidi.

These are all by some regarded as identical with the common pathogenic form *Strep. pyogenes*. Heinemann. Jour. Infec. Dis., IV., p. 87, 1907.

distinctive feature of which is the rather dense red colony, with a series of very slight projections around the edge, so that

the colony looks almost burr-like. These projections are very short and not always found; but when they are found, it is practically certain that the colony represents the *Bact. lactis acidi*. It should also be borne in mind that no bacterium that grows on the surface of the plate is of this type, for this organism grows only under the surface.

FIG. 16—COLONIES OF
BACT. LACTIS ACIDI
IN LITMUS GELATIN



Characteristics of Bact. Lactis Acidi.—This organism is of so much importance to dairymen that it is necessary to study it in more detail, and the following description of the growth of this organism and the various culture media used in the laboratory will serve to identify this type. Its growth *on the surface* of any culture medium is extremely scanty. No growth can be seen on *potato*, and none on the surface of gelatin plates. When inoculated on *agar slants* there is an extremely thin growth, almost invisible, although it may be seen, by very careful examination, as a clear, transparent film. On the other hand, it grows fairly well in the depth of culture media away from contact with air. Inoculated by a needle, into a *gelatin tube*, it grows well below the surface along the line of inoculation (Fig. 17), but it stops at the surface and never spreads over it. When inoculated into ordinary *bouillon*, usually no visible signs of growth are seen, although the organism does grow slightly. Inoculated into *fermentation tubes* containing bouillon with milk sugar or dextrose, there is always a scanty growth giving rise to slight turbidity, and growth may extend up the closed arm of the tube, as well as in the open arm, indicating that the organism grows without contact with oxygen (*Facultative anaerobic*).

The most interesting characteristic is its effect upon milk. When inoculated into sterilized milk, it grows readily and, in a short time, begins to produce acid. The rapidity of the acid production is variable with different cultures. It is so rapid in some cases that, if the specimen be placed at body heat, it will curdle in six hours. With other cultures, the curdling under similar circumstances would not occur for twelve hours, or three days, and with some cultures curdling never occurs. All specimens of milk, however, become acid, although not always sufficiently to precipitate the casein. Between these extremes every conceivable grade may be found in cultures that are, in other respects, identical; and they represent, doubtless, one type, differing in its power of producing acid.

The power of producing acid not only varies in the different samples of this type, but in different cultures from the same original source. It increases if the bacteria are cultivated for a while in milk. If, for example, a culture is taken directly from sweet milk, it will usually curdle the milk slowly; but if daily transferred from one culture of milk to another for a few days, and then taken out and tested, its power of producing the acid is found to have much increased. Since this power thus varies in successive cultures from the same original source, it would seem that the fact that

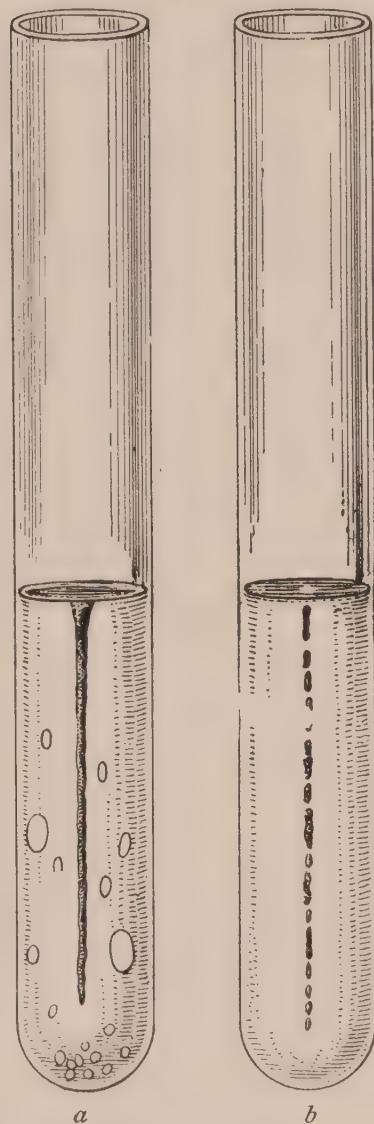


FIG. 17—GELATIN STAB CULTURES

- a. *Bact. lactis aerogenes*
- b. *Bact. lactis acidi*

some of the cultures found in a sample of milk will sour and curdle it quickly, while others do not, only indicates that we are dealing with varieties of the same bacterium, and not with different species.

To this type belong the largest number of bacteria known to cause the common souring of milk. Most of the butter starters (see page 203) are cultures of this class and the same is true of most of the cheese starters. Several different types of lactic acid bacteria have been described by Freudenreich as associated with the ripening of Swiss cheese. These, as he studied them, showed considerable difference in their action on cheese. Some appeared to benefit the cheese, while others did not. They showed other differences also, but most of them belong to this same general type of bacteria. This emphasizes the fact that the *Bact. lactis acidi* represents a *group of varieties, and not a single organism*. If the question is asked whether it represents a single *species*, or a collection of species, the answer is, that no one knows what is meant by the word species among bacteria, and hence, this question cannot be answered. It certainly represents a collection of forms related to each other, but showing some noticeable variations.¹

The type of milk curdling produced by this organism is quite easily recognized. The milk becomes strongly acid, and turns into a hard curd, without any trace of gas bubbles, and without the separation of any whey. (Fig. 18, B.) The milk simply becomes one smooth, solid curd, with a clean, sharp, sour taste, and no odor. This type of curdling has been recognized as a desirable one by the dairyman, since it is most favorable for dairy processes, and is consistent with the production of the best grades of butter and cheese. This organism grows readily at temperatures from 60° to 100°, growing more rapidly at higher temperatures. At a temperature of about 70° it

¹ Müller. Cent. f. Bact., II., xvii., p. 468.

Löhnis. Cent. f. Bact., II., xviii., p. 97, 1907.



FIG. 18—EFFECT OF DIFFERENT BACTERIA ON MILK

A. *Bact. aerogenes*. B. *Bact. lactis acidi*. C. & D. Liquefying bacteria

grows with great rapidity, and at this temperature it seems to be more vigorous than any other bacterium ordinarily found in milk. For this reason, as we shall presently see, milk kept at 70° becomes, in a short time, almost completely filled with this species of bacterium at the expense of all the others that might have been there originally. This type of organism is the dairyman's friend.

BACTERIUM AEROGENES

The second type of lactic acid bacterium differs in many respects from the first.¹ It is not so common as the first type, but is, nevertheless, very widely distributed, and is very likely to be present in any sample of milk in some quantity. If it is present in large quantities, it becomes a very great nuisance; if only a few are present, it may do no injury. The organism in question can commonly be distinguished by a study of the

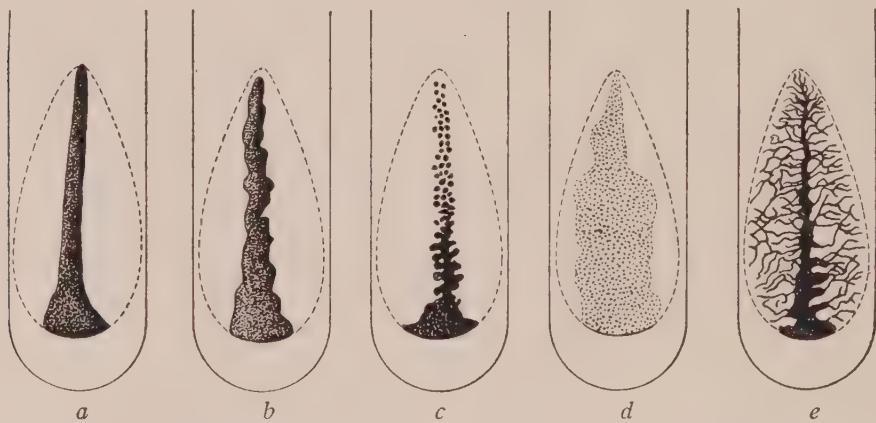


FIG. 19—TYPES OF BACTERIAL GROWTH ON AGAR SLANTS
 a. Filiform. b. Echinate. c. Beaded. d. Effuse. e. Arborescent

gelatin plates described on page 288 by the fact that it produces a strongly acid colony which grows *on the surface* of the gelatin as a round, more or less raised mass, spreading sometimes to the size of 2 or 3 mm.

Bact. aerogenes, when examined with a microscope, is seen to be an organism somewhat larger than the other lactic type, and one which is, beyond question, a Bacterium, being considerably longer than it is broad. Its growth on various laboratory media is very easily distinguished from the first lactic acid type. When inoculated on *the surface* of agar tubes, or potato, it grows luxuriantly, forming a thick, whitish layer. In *gelatin tubes* it both grows along the needle inoculation and

¹ Dominikiewicz. Milchzg., p. 817, 1903.

Baginsky. Zeit. f. Phy. Chem., xiii., p. 434, 1889.

spreads over the surface. (Fig. 19, *b.*) In *bouillon* it grows luxuriantly, producing a turbidity and sediment. Inoculated into fermentation tubes containing sugar, it grows both in the open and closed arm, and in all cases produces gas which collects at the top of the closed arm. (Fig. 20.) This latter is the most important character of this type. When inoculated into milk, it causes the souring of the milk, which is rapidly followed by curdling, the rapidity of the curdling varying in different specimens. The curd which is produced differs very much from that of the first type of lactic acid bacteria. It is always more or less filled with gas bubbles, and when care is taken to obtain a typical curd, it appears, as shown in figure 18, *A*, crowded with holes which represent the bubbles of gas formed by the organism. The whey commonly separates in a short time from the curd, and the final appearance is strikingly different from that of the curdled milk produced by the first type of lactic acid bacterium. There is also a difference in the type of lactic acid produced by the two organisms, the first producing what chemists refer to as *right-handed lactic acid*, and the second producing chiefly *left-handed acid*, two terms referring to the effect of the acid upon polarized light.¹

This organism plays a great deal of mischief in dairying.

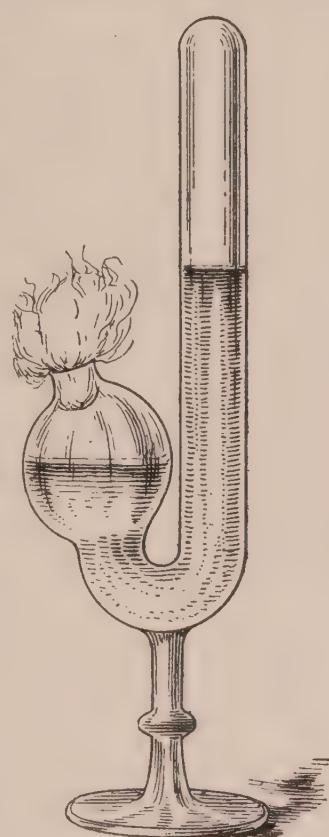


FIG. 20—PRODUCTION OF GAS IN FERMENTATION TUBE, PRODUCED BY BACT. LACTIS AEROGENES

¹ Utz. Cent. f. Bact. II., xi., p. 600, 1904.

Thiele. Zeit. f. Hyg., xlvi., p. 395, 1904.

Heinemann. Jour. Biol. Chem., II., p. 603, 1907.

The production of gas is the cause of the ruin of vast quantities of cheese. If milk contains a considerable quantity of this bacterium, instead of the more common non-gas-producing type, when it is made into cheese, the bacteria grow and develop gas, the cheese becomes filled with the bubbles, swelling more and more, until it finally results in what is known as swelled cheese. At the same time that this swelling occurs, the flavor of the cheese becomes unsatisfactory, so that the swelled cheese is practically worthless, at least in a case of extreme swelling. These organisms have been the cause of the loss of enormous amounts of money on the part of cheese makers. In butter making they are not so disastrous, but here, too, their presence is undesirable, for they sometimes produce unpleasant flavors in the creams, resulting in an inferior grade of butter. This type of organism, therefore, is decidedly the dairyman's foe.

Varieties of Gas-producing Bacteria.—*Bact. acrogenes* has been mentioned above as a single type, but there is a long list of bacteria capable of developing acid-producing gas, and growing, generally, much like the one which we have described.¹ These have been separated into several groups, with several names. The dairyman cannot expect to separate the different types from each other, and it is not appropriate in this work to attempt to describe them. They may all be considered together, therefore, as the type of gas-producing *Bact. lactis aerogenes*. One special type, however, which in most respects agrees with the one described above, must be specially noticed. This one also produces acid and gas from milk in the same method as the *Bact. aerogenes*, and its growth on potato, agar and elsewhere agrees in almost all respects with it. But it is found to be *motile*, and covered with a number of flagella. It is, therefore, not a Bacterium, but a Bacillus. The organism in ques-

¹ Harrison. Cent. f. Bact. II., xiv., p. 359, 1905.

Conn. Ann. Rep. Storrs. Exper. Sta., 1906.

tion, though not so common as the others, is yet not infrequently found. It is of some importance, however, because it is generally recognized as indicating the presence of fecal contamination, and is known to bacteriologists by the name of *B. coli communis*. This is a type of Bacillus found living in the intestines of animals, and is not found in any abundance in nature, outside of the intestines. When, therefore, any water is found to contain this organism in any quantity, it is a fairly safe indication that the water is contaminated with sewage. If bacteriologists, in their study of drinking water, discover the presence of this organism they conclude that the water is contaminated with sewage and, hence, unsafe to drink; not because *B. coli* is in itself dangerous, but because it indicates sewage contamination.

What about the presence of this organism in milk?

In the first place, if the presence of *B. coli* in milk should result in its being pronounced unfit for drinking, a large proportion of the milk in our markets would be under ban, for, while it is not by any means universal in milk, still it is found with very great frequency. Indeed, it is quite evident that one would naturally find it in milk. Anyone who knows the conditions under which milk is produced, even in the best and most cleanly dairies, will easily recognize that there is always a possibility of the milk being contaminated with animal excreta. This excrement is almost sure to contain *B. coli*; hence, it will follow that the milk will very likely show its presence from such contamination. It may, therefore, be fairly safe to assume that the presence of this bacillus in milk indicates the contamination of the milk with animal fæces; but it by no means follows that this should be sufficient to condemn milk for drinking purposes. It is true that water contaminated by sewage is unsafe to drink, because it contains excreta from human bodies and is, therefore, quite likely to contain germs of human diseases, chief among them typhoid fever. Anything con-

taminated by the typhoid germ is dangerous. But the cattle which produce milk do not suffer from typhoid fever and, with the exception of tuberculosis, which will be especially mentioned later, no diseases found in the intestinal canals of animals are likely to be distributed to man. Hence, while a small quantity of animal faeces in milk may not be an appetizing matter to think of, it does not necessarily, or even probably, render the milk at all likely to distribute infectious diseases to those drinking it. Thus it follows that, although the presence of *B. coli* may render water unsafe, because it suggests sewage contamination, its presence in milk does not render the milk unsafe, but merely indicates that there may have been a certain amount of contamination with animal faeces. This fact is of universal occurrence, as any one knows who has ever visited a cow stall. *B. coli* is not harmful in itself, and animal faeces are by far less dangerous than human excreta. Clearly the presence of *B. coli* in milk has a very different meaning from its presence in water.

Other Types of Lactic Bacteria.—The two types of bacteria described are by far the most important of the acid bacteria of milk. Each of these, as indicated, represents a type containing many varieties rather than a single species. In addition to these there are other bacteria that produce lactic acid not strictly belonging to either of these types. Some do not produce any gas, but grow quite abundantly upon the surface of culture media, sometimes producing small, and at other times quite large, colonies. It is impossible in this work to attempt any description of these forms. Although there are numerous varieties of lactic bacteria, in the vast majority of cases milk is soured by one of the two types above mentioned. Whether the one or the other of these types will be found to cause the spontaneous souring in any sample of milk cannot be predicted; for it is partly dependent upon temperature. If the milk has been kept at 70° or lower, it will, in most cases, be soured by

Bact. lactis acidi, but if it has been kept at 80° or above, it is more likely to be soured by the *Bact. aerogenes* type. Milk soured at high temperatures therefore commonly contains left-handed lactic acid, while that soured at low temperature contains right-handed acid.¹ These facts are of special importance in cheese making as will be noted later.

PEPTONIZING AND RENNET FORMING BACTERIA

At times the dairyman is puzzled by a somewhat unusual phenomenon, for he finds his milk curdling, but not becoming sour, and he speaks of the phenomenon as "sweet curdling." It is apt to occur in the fall or spring, when the food of the cattle is being changed. It is due to a class of bacteria that have the power of secreting certain chemical ferments very similar to those produced by the digestive glands of the stomach and intestine.

Upon almost any gelatin plate made from milk (see page 280) there will be found, in addition to the colonies of lactic acid bacteria, a number of pits in which the gelatin is being dissolved or liquefied. This effect is produced by bacteria, and if a platinum needle be dipped into one of these pits and then

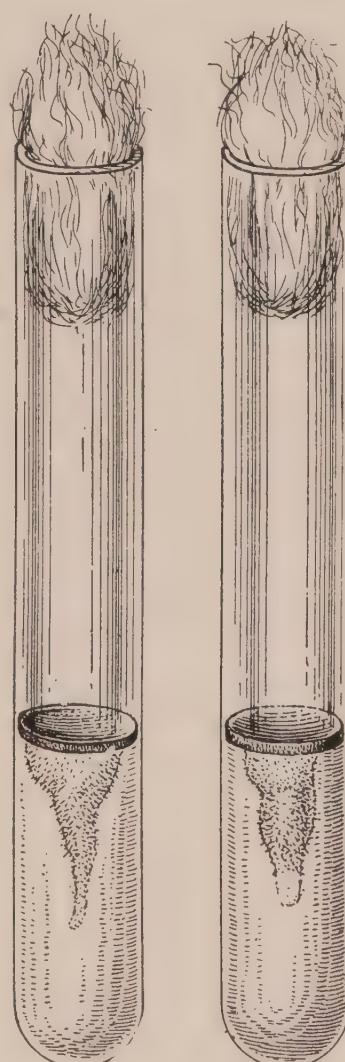


FIG. 21—LIQUEFACTION OF GELATIN BY ENZYME-FORMING BACTERIA

¹ Ferguson. Die spontane Zersetzung der Milch. Göttingen, 1902.
Conn. Ann. Rep. Storrs. Exper. Sta., 1906.

stabbed into a gelatin tube, in a day or two it will be found that the bacteria thus inoculated are liquefying the gelatin, as shown in figure 21. These bacteria do not liquefy agar, and can, therefore, be best studied in gelatin.

If these bacteria be inoculated into milk, they will usually curdle it in the course of a day or two; but they do not produce any acid, hence they act differently from the lactic acid bacteria. The curdling is actually brought about by a chemical ferment secreted by the bacteria, which is very similar to *rennet* secreted by glands in the stomach of mammals, the material used by cheese makers to curdle their milk. Such chemical ferments are called *enzymes*; and it is an enzyme secreted by this class of bacteria which causes sweet curdling.¹

But these bacteria produce a second action, since liquefaction of gelatin is also produced by an enzyme, though not by rennet. The enzyme in question is quite similar to another digestive ferment in the stomach, and also somewhat like *pepsin*. It has the power of converting casein and other proteids into soluble products, prominent among which is *peptone*, which is a sort of soluble proteid. Hence, this class of bacteria is frequently called the *peptonizing bacteria*. They are also called *liquefiers*. Since many of them are putrefactive in their action, they may, in the end, fill the milk with putrefactive products.

Thus, this class of bacteria actually secretes two different enzymes, or ferments. The first one curdles milk, much as rennet does, the second softens the casein and converts it into rennet-like bodies. Hence, when they are inoculated into sterilized milk, the milk first curdles, the curd being rather soft and usually not acid. After a few days the curd begins to show

¹ Fermi. Cent. f. Bact. x., p. 401, 1891; xii., p. 713, 1892.

Conn. Cent. f. Bact. xii., p. 223, 1892.

Lubbert. Zeit. f. Hyg. xxii., p. 1, 1896.

Duclaux. Le Lait., 1887.

signs of softening, because it is being converted into peptones, or other soluble substances. As these are formed, they are dissolved and the curd disappears. Since this is a process very similar to digestion of milk, in the stomach and intestine, it is customary to say that the curd is *digesting*. This may continue until the whole curd is dissolved into a watery liquid, or it may stop after it is only partly dissolved. (Fig. 18, *C* and *D*.) With some species of this class the digestion occurs without any previous curdling, the rennet action being slower than the digestion. In these cases the milk simply becomes more transparent, and analysis shows that it has digested much the same as in the other cases where the curdling first appears.

The liquefying bacteria are very numerous and are almost always found in samples of fresh milk. They are of no great importance in the ordinary handling of milk since they are commonly overshadowed by the lactic acid bacteria. Though they sometimes produce a sweet curdling, they rarely develop sufficiently in milk to show signs of the digestion just described. Occasionally, however, they are of significance in cheese making. During the long ripening of cheese they have a better chance to grow than in milk. Whether they have much influence upon the hard cheeses is not fully known yet, but in the ripening of soft cheeses, they sometimes multiply so extensively as to produce very bad results, and to cause much loss to the cheese makers. While, therefore, they are of little importance to the one who handles milk, they play a considerable part in the making of cheese. Some instances have been described where this class of bacteria seems to be particularly abundant and develops instead of the lactic type. The trouble has been attributed to the bacteria in the udder, where they are thought to be especially abundant in the case of incomplete milking.¹

¹ Burri. Milchzg., v. 705, 1903.

Bacteria Producing Both an Acid and a Ferment.—The peptonizing bacteria usually fail to produce acid and, indeed, make the milk slightly alkaline. Among the acid forming bacteria are some which produce a peptonizing enzyme, like those just mentioned, but they produce acid at the same time. This type is thought by Gorini to have an important part to play in the ripening of some cheeses¹; but there is no sufficient proof of this at present. One variety of this class has been found to be the cause of a bitterness in some types of cheese, bringing great losses upon the dairymen. They make the milk sour and are not, therefore, readily detected in normal milk. They can easily be found by the ordinary litmus gelatin plate methods.

NEUTRAL TYPES

A large number of milk bacteria apparently play no part in dairy problems, and, therefore, we need not consider them except by a passing word. Upon studying the litmus gelatin plates (page 289), we will find small colonies that are not acid, that do not liquefy the gelatin, but appear simply as whitish or greyish dots, some on the surface and others under the surface of the gelatin. If we will remove and study these by themselves, we will find most of them to be *neutral* organisms. They do not sour or curdle the milk, nor do they produce any changes in it that are noticeable under any ordinary conditions. Neither do they seem to have any pathogenic effect upon persons drinking the milk, although they may be present in considerable quantities. Up to the present time, therefore, we have no reason to regard them of any special significance to dairymen. We need consider them no farther, beyond stating that they are frequently present in considerable numbers.

¹ Gorini. Cent. f. Bact. II., viii., p. 137, 1902.

BUTYRIC ACID FERMENTATION

The presence of *butyric acid*, accompanied by a rancid odor, is not uncommon in milk products. It is well known in samples of butter and, once in a while, is found in old samples of cheese. In milk, however, butyric acid fermentation rarely occurs. It

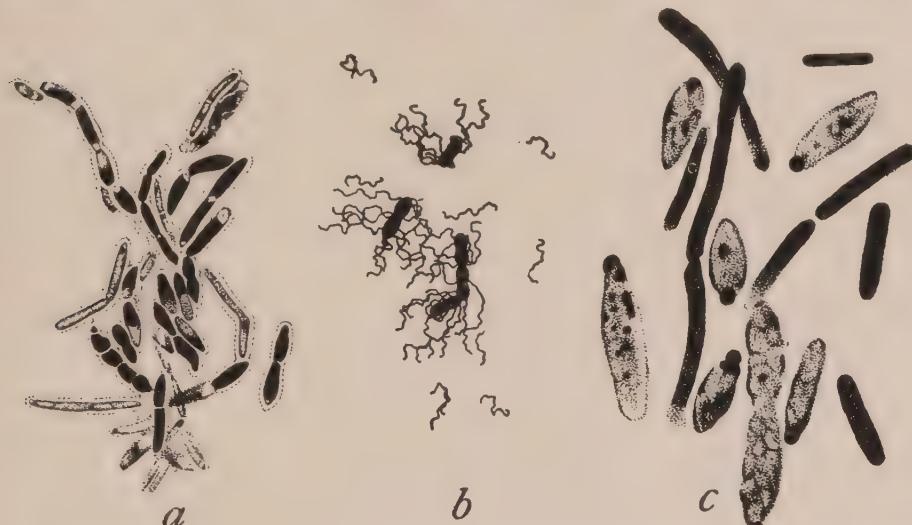


FIG. 22—BACTERIA OF BUTYRIC ACID (SHATTENFROH AND GRASSBERGER)

is sometimes produced by certain types of bacteria (Fig. 22), which are practically always present in milk, although they rarely have an opportunity to develop.¹ These organisms are commonly anaerobic, and will not develop in milk kept under ordinary conditions. Moreover, the rapid growth of lactic bacteria in milk, in almost all cases, prevents the butyric acid germs from developing at all. For these reasons, this fermentation does not occur in ordinary milk, and in the common bacteriological study of milk, butyric bacteria do not appear. They can, however, usually be found by a simple device, since the most common types of these organisms are spore producers and are, therefore, not killed by ordinary boiling temperatures. If milk be boiled for a few moments, and then plates be made from

¹ Kedroski. Zeit. f. Hyg. xvi., p. 445, 1894.

Schattenfroh and Grassberger. Arch. f. Hyg. xxxvii., p. 54, 1900; xlii., p. 219, 1902; lx., p. 40, 1907.

it, the spores will develop. (See page 299.) If these plates be cultivated under anaerobic conditions, the butyric bacteria will have a chance to develop and will produce colonies in the usual way, which subsequently may be isolated and studied. But the study of anaerobic bacteria is too difficult for the beginner. Moreover, these organisms are of so little importance in dairy problems that it is not worth while to consider them further here. The only conditions under which they become of dairy importance is in the samples of old butter that they have turned rancid, and this phenomenon is hardly important enough to deserve here our special attention.

ABNORMAL MILK BACTERIA

Under this head is included a variety of bacteria which produces unusual fermentation in milk, and occasionally gives rise to important dairy troubles. They are by no means always found, and, by proper care, they may be completely avoided. Some of those listed below are very rare and are placed here for convenience' sake only; others, however, are quite common.

Slimy Milk.¹—This is one of the commonest of troublesome dairy infections. There are two different types. In some cases the milk is found to be slimy at the time it is drawn from the cow. Such a trouble is due to a diseased condition of the cow's udder, and is generally attributed to *garget*. In such a case it is frequently accompanied by bloody milk from the diseased udder. Such milk should always be excluded from the dairy. In fact, no milk that is bloody or slimy when drawn

¹ Adametz. *Landw. Jahr.*, p. 185, 1891.

Marshall. *Bul. 140, Mich. Exper. Sta.*, 1897.

Ward. *Bul. 165, Cornell Exper. Sta.*, 1899.

Konig. *Speckermann and Tillman's Rev. Gen. d'Lait*, ii., p. 115, 1902.

Gruber. *Rev. Gen. d'Lait*, ii., p. 97, 1902.

Harrison. *Rev. Gen. d'Lait*, v., p. 73, 1905.

Burri. *Cent. f. Bact.*, II., xii., p. 371, 1906.

Weigmann. *Milch. Zent.*, i., p. 5, 1905.

from the cow should be used for food purposes. Gargetty milk is not caused by bacteria.

The other type of slimy milk is due to the growth of bacteria. The sliminess in this case is not apparent when the milk is drawn, or for a number of hours afterward; but by the time the milk is from 12 to 24 hours old, depending upon the temperature, there begins to be evident an unusual change; instead of souring in the normal way, the milk becomes more or less viscous and, finally, may be extremely slimy, so that it may be drawn out in long threads which, when dipped out with a dipper, will run down in long streams. (Fig. 23.) Such slimy milk is apt to be counted as a total loss, for it is usually regarded as absolutely worthless. But there is no reason to think that the milk is really unwholesome. Indeed, in

Norway the people are fond of such milk and frequently use artificial means to produce it, preferring slimy milk to the ordinary kind. In this country slimy milk is thrown away, or fed only to pigs. Since the sliminess does not appear until the milk is several hours old, no loss ensues when the milk is used immediately. Only milk distributed to consumers or kept for a day or so will be thrown away because of sliminess.

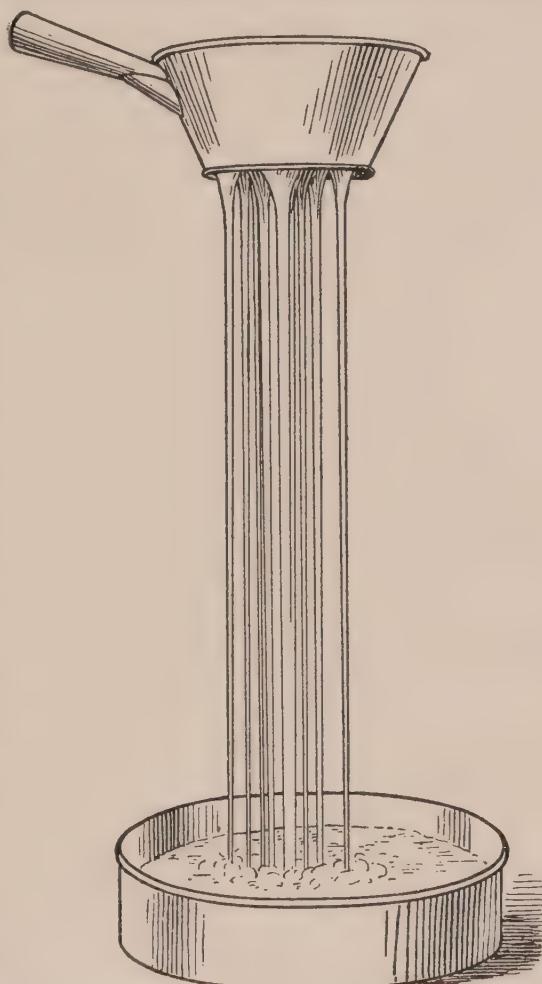


FIG. 23—EFFECT OF SLIMY MILK BACTERIA UPON MILK (RUSSELL)

The cause of such sliminess is, so far as known, the growth of certain types of bacteria. Several different kinds have been found capable of making milk slimy. The most common one of these seems to be *Bact. lactis viscosus* (Fig. 24), which was first described in Europe, and has later been found in a number of such infections in America. This organism is widely distributed but, unless present in great numbers at the start, it does not, as a rule, develop in milk, since the growth of lactic bacteria prevents its becoming very abundant. Although sev-

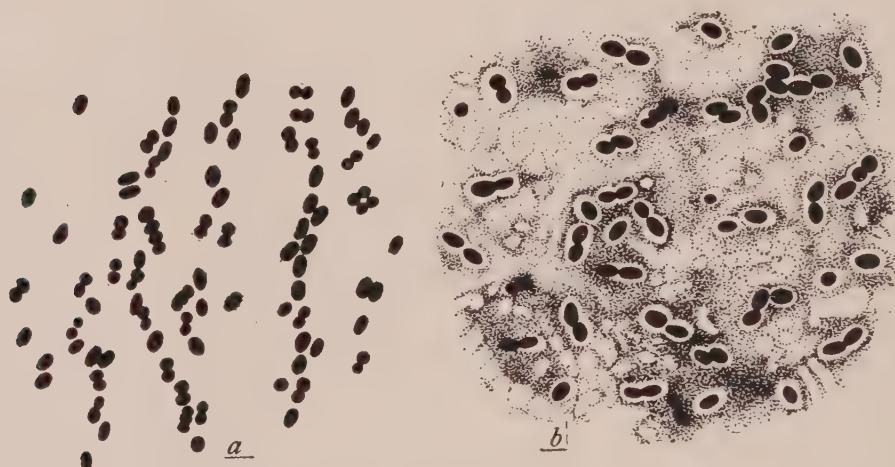


FIG. 24—*BACT. LACTIS VISCOSUS*, THE COMMON CAUSE OF SLIMY MILK (WARD)

eral other species of bacteria are able to produce slimy milk they are not commonly causes of dairy troubles. Some varieties of *Bact. lactis acidi* and *Bact. aerogenes* produce slimy milk.

A slimy milk infection is sometimes persistent, remaining in dairies for weeks, and even months, and may be distributed from one dairy to another until a large community is more or less infected with the same trouble. One of the common methods by which a large community may become infected with such a trouble is through a central creamery or milk distributing station. The trouble begins in some one dairy, and the milk

from this dairy is carried to a creamery or station; from here the cans which brought it are freely distributed to other dairies, and, in time, nearly every dairy in the community will receive these cans which may have been partially washed in the creamery, but never thoroughly sterilized. By these means the germs present in the cans are readily distributed and the sliminess begins to appear in one dairy after another. The remedy for such cases is clear. A more thorough washing and sterilizing of the cans at the central distributing station must be adopted so that no farm will ever receive a can containing any of the undesirable bacteria. In this way the spread of such an infection may be checked.

In all cases there must be some original source from which the trouble arises, and this source must be looked for carefully and, when found, eliminated if possible. Studies of such infection have shown, at least, three possible sources. 1. Sometimes the germs have been obtained from the water used in washing the cans or, more likely, from the water in which the cans were cooled. The presence of the slimy bacteria in the cooling water will surely, in time, infect the inside of the cans, when they will bring about the trouble. 2. Sometimes it has been found that the udder of a single cow has been infected with the germ, and this has affected the milk of the whole dairy. 3. The slimy milk germ has also been found in the dust of the food which has been fed to the animals.

No other sources, as yet, are known. Hence, the dairyman who finds this trouble appearing in his dairy should look to his water supply, to the food of the cows, and should, for a few days, keep separate the milk from the individual cows to see whether the sliminess of the milk affects the entire herd, or only one or two cows. As soon as this is determined, he will know whether he needs to clean the udders of one or two cows, or to find some more general source of infection. It is impossible here to give directions how to discover and remove the cause

of such infection; for conditions vary with different dairies; but generally, when an intelligent dairyman has discovered the source of the infection, the remedy will suggest itself.

BITTER MILK

The appearance of a *bitter* taste in milk is a rare trouble for the milkman, though a common one for the cheese maker. The causes of the bitterness are, at least, three. 1. Sometimes it happens that the milk has a bitter taste as soon as it is drawn from the cow. In such a case it must be attributed to

some food which the animal has been eating. It is known that if the cattle feed upon lupine or ragweed, a certain amount of bitterness develops in the milk. The remedy is simply to change the food of the cow. 2. Sometimes it happens that milk tastes perfectly sweet when freshly drawn, but, in the course of a few hours—sometimes not for a day or two—a bitter taste develops in the milk. Here the taste is due

to the growth of *bacteria* in the milk (*B. liquefaciens amari*, *M. casei amari*), which produce certain bitter products.¹ This type of dairy trouble is quite rare, and only two or three examples of it have been carefully investigated. (Fig. 25.) But bitter tastes in cheese, due to a similar cause, are common. Two different bacteria have been found to give rise to the bitter tastes. The source of the organisms was traced, in one case, to the udders of certain cows, but in other cases the source was not found. One extensive series of bitter milk infection was found in a cheese factory in Canada, and produced a great deal of trouble. In this case, the cause of the bitterness

¹ Conn. Cent. f. Bact., ix., p. 653, 1891.

Bleisch. Zeit. f. Hyg., xii., p. 81, 1893.

Hugge. Rev. Gen. d'Lait., v., p. 470, 1906.

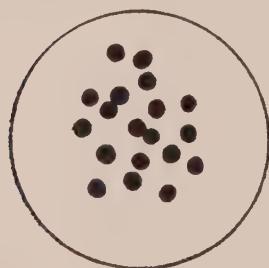


FIG. 25—COCCUS PRODUCING BITTER MILK (CONN)

was traced to a *yeast* which had made its way into the dairy supplying the factory, and which was producing the mischief.¹ (Fig. 26.) This bitter yeast was found on the leaves of trees under which the open cans of milk were allowed to stand. In all such cases the only remedy is, to trace the trouble to its source, so as to be able to prevent a recurrence of the trouble, and then to clean thoroughly the udders of the cows and disinfect all milking utensils—cans, vats, etc. 3. The third type of bitter milk is of less importance to the dairyman. It occurs in milk which has been subjected to heat for the purpose of sterilizing it. Such action destroys most of the bacteria present, but sometimes leaves alive some of the spore-bearing organisms. When such milk is kept for some days, although it will not sour and usually will not curdle, it does undergo other changes, and frequently becomes bitter. The bitterness may increase in strength with each day, making the milk very unpleasant to the taste. Inasmuch, however, as the use of sterilization is not a common process in dairying, and sterilized milk is rarely kept for any length of time, this type of bitter milk is of very little significance. There have been instances where factories that put up a sterile milk have experienced considerable loss from the development of bitter organisms in their product. Their only remedy is found in more complete sterilization, by applying higher heat, which will kill all spores.

Alcoholic Fermentation.—Although some forms of sugar



FIG. 26—*TORULA AMARI*, PRODUCING BITTER MILK AND CHEESE (HARRISON)

¹ Harrison. Ontario Agr. Coll., 120, 1902.

readily undergo alcoholic fermentation, this is not true of milk sugar. Fruit juices are quickly acted upon by yeasts, and converted into Carbon dioxide and alcohol. Milk sugar, although readily affected by bacteria, is not easily acted upon by yeasts, and does not normally undergo alcoholic fermentation. The reason for this has to do with the chemical nature of the sugar molecules, and does not concern us here. It is very important to remember, however, that when solutions of fruit sugar are left exposed to the air, they ferment, giving rise to alcohol; but when milk sugar is exposed to similar conditions, lactic acid is produced, but never alcohol.

It is, however, possible to produce an alcoholic fermentation in milk by adding thereto a little cane sugar and then inoculating it with yeasts.

This fact has been learned by manufacturers of condensed milk to their sorrow. The common brands of condensed milk are preserved by the addition of a large amount of cane sugar. Since bacteria cannot thrive upon cane sugar, it serves as a partial disinfectant for the milk. But although bacteria will not grow in condensed milk, yeasts are capable of growing there, and of fermenting the sugar. It sometimes happens that yeasts get into the milk, and the process of condensing does not kill them. When the milk is subsequently sealed in the cans as condensed milk, the living yeasts are inclosed. They find the sugar a medium favorable to growth, and an alcoholic fermentation occurs. Immense losses have at times occurred as the result of such growths. To prevent this loss, the process of condensing and final sealing must be carried on in such a way as to avoid yeast contamination, or the yeasts must be destroyed by heat.

The possibility of making milk undergo an alcoholic fermentation by the addition of yeasts is made use of in the manufacture of *kummys*.¹ This beverage was originally prepared by

¹ Schipin. Cent. f. Bact., II., vi., p. 775, 1900.

the Arabs from mare's milk, which will undergo an alcoholic fermentation; but now an imitation product is widely made and used, prepared from cow's milk. A small quantity of sugar is added to milk, and some common baker's yeast. An alcoholic fermentation soon begins, and the fermented product is kummys. Various modifications of this general process are adopted by different makers, for kummys has become a commercial article.

Several other alcoholic beverages, in different countries, are made by special methods from cow's milk.

The first of these to which attention was given is *kefir*, a beverage long known in the Caucasian mountains, and produced from ordinary milk by adding some bodies, called *kefir grains*. (Fig. 27.) These grains have been in the possession of the people from time immemorial. They are placed in milk, where they soon produce a fermentation. After the fermentation is over, they are removed and used in some more milk, or, perhaps, they are dried for preservation for future use. Kefir grains consist of a mixture of several kinds of bacteria and of yeasts.¹ The nature of their action is essentially a double one. The bacteria have, first, a chemical action upon milk sugar, converting it into a type which is easily fermentable by yeast, and the subsequent alcoholic fermentation which occurs, is due to the



FIG. 27—KEFIR GRAINS

¹ Freudenreich. Land. Sahr, d. Schw., 1896.

action of the yeast upon the modified milk sugar. The beverage that is produced contains alcohol, lactic acid, and certain other flavors, which are produced by the bacteria action on the milk.

Other types of fermented milk beverages are found in various countries. One called *leben*¹ is common in Egypt. *Mazun*² is made in abundance in America. The Turkish people have one which they call *yoghourt*.³ In Sardinia an alcoholic milk beverage is called *goiddu*.⁴ In all of these cases the beverage is made by ferments which the people prepare by special methods and keep on hand. In many places the ferment is simply a little of the old fermented milk inoculated into the new milk. Most of these forms of alcoholic milk have been studied by bacteriologists and have been found to be based, in general, upon the same principle. They all represent a combined action of bacteria and yeasts. The bacteria, in all cases probably, first change the nature of the milk sugar, so that it is fermentable, and the yeast subsequently changes the sugar into alcohol and carbon dioxide. These beverages are frequently regarded as more easily digestible than ordinary milk, and they certainly have a more pleasant flavor. Perhaps their popularity is due simply to the fact that they contain alcohol.

FERMENTATION CHANGING THE COLOR OF MILK

The first type of fermentation ever studied in milk was *blue milk* which, even 60 years ago, was found to be a more or less common dairy trouble in certain sections of Europe. The phenomenon appears as follows:

The milk is perfectly normal when drawn, and for several hours afterward, but at about a time when it begins to become sour, blue spots appear in it, which extend through the milk

¹ Rist and Khoury. Ann. d. l'Inst. Past., xvi., p. 65, 1902.

² Duggeli. Cent. f. Bact., II., xv., p. 577, 1905.

³ Zeit. f. Fleisch u. Milch Hyg., xv., p. 248, 1905.

⁴ Grisconi. Milch. Zent., II., p. 425, 1906.

until the whole finally becomes a deep blue color. The cause of the phenomenon was studied in 1840 and found to be a bacterium, to which the name *Bacillus cyanogenes* was given.¹ This bacterium has been found many times since, and is one of the well known dairy organisms. (Fig. 28.) It is not very common, however, and dairy infections due to blue milk are extremely rare. The organism has been found in Europe and America, also, but dairy infection of blue milk has seldom been reported from American dairies. It is very easy, however, to produce blue milk by inoculating with the bacteria in question, but it does not seem to have been of common occurrence enough to have caused much trouble in this country.

Red Milk.—A red color in milk is not an uncommon phenomenon. Often it is because the milk is bloody, through some wound in the udder. Sometimes cows give red milk after eating madder, or some other plants. In all these cases the trouble shows itself at the time of milking. But, in addition to such causes, red milk is sometimes due to bacteria, although as a dairy phenomenon, this is of very rare occurrence. When it does occur, the milk is generally red only on the surface. Several bacteria are known to make milk red, among which are: *B. prodigiosus*, *Bact. erythrogenes* and *B. lacto rubificiens* and others. They are fairly common in milk, but rarely do they cause any trouble.²

¹ Fuchs. Mag. f. d. ges. Thierh., 1841.

² Baginsky. Cent. f. Bact., v., p. 448, 1889.

Gruber. Cent. f. Bact., II., viii., p. 457, 1902.

Gratz. Milch. Zent., i., p. 9, 1905.

Menge. Cent. f. Bact., I., vi., p. 596, 1889.



FIG 28—*B. CYANOGENES*

Other Colors in Milk.—Several other bacteria are capable of producing color in milk. Some turn it *yellow*, some *green*, some *brown* and some *black*. By choosing proper species of bacteria, fermentations can be produced in milk, showing all the colors of the rainbow. But these phenomena, although found in the laboratory, and of great scientific interest, do not appear in practical dairying. Some of these colors occasionally appear on soft cheeses that are ripening, but they do not occur in milk. For these reasons we shall not here consider them further.

MILK HAVING THE TASTE OF TURNIPS

Milk is sometimes found to develop a turnipy taste, a fact which, until recently, has been attributed to cows eating turnips, or some similarly tasting food. In some cases this is probably the correct explanation; but a careful study of the phenomenon has recently shown that some of their troubles are due to the growth of bacteria in the milk. If the taste is directly due to the food eaten by the cows, it will appear in the milk when the milk is drawn. But in some cases this turnipy taste does not occur until several hours after the milk has been drawn; consequently it must be due to the growth of micro-organisms.¹ Bacteria producing this turnipy taste have been separated from milk and, by experiment, have been shown to produce this turnipy taste (*B. lactis fætidus*). The dairyman, therefore, must remember that this trouble may be due to the food, or there may be a bacteria infection. He can easily determine which it is by noticing whether the taste is present when the milk is drawn, or develops some hours afterward. In the latter case he may get rid of it by enforcing greater cleanliness, even though he still feeds turnips to his cows.

¹ Gruber. Hyg. Rund., p. 626, 1903.

Ritland. Zeit. f. Fleisch. u. Milch. Hyg., xiv., p. 276, 1904.

SOAPY MILK

A somewhat peculiar infection in milk has been discovered in one or two cases in Europe, and has been called soapy milk; but it has not, so far as is known, occurred in America. The milk in question develops a slight soapy taste which is decidedly unpleasant. Two different species of bacteria (*Bact. sapolacticum* and *B. lactis saponacci*) have been isolated from such milk, and have been proved to produce the trouble. The phenomenon is of no special significance, at least in American dairies.¹

OIDIUM LACTIS

This organism is very widely distributed and plays an important part in certain dairy processes, although it has very little or no relation to milk used as milk. It differs from other bacteria in appearance and method of growth (Fig. 29), and seems to be about half way between a bacterium and a mold. Like molds, it grows generally upon the surface of liquids and solids, and does not, like bacteria, penetrate into their depths. When growing upon the surface it produces long, delicate threads, which radiate from a central point where the growth starts and extends over the surface of the medium for considerable distances. At first it looks exactly like a mold, but, after growing for about two days, these threads break into a large number of very short segments, which serve as spores; for they will grow and generate into fresh threads, if planted upon favorable places. The organism grows with extreme rapidity, and when planted on the surface of hardened milk, like a soft cheese, it will spread over the surface so rapidly that, in the course of a very few hours, it may cover the whole surface. For this reason, it is found to be one of the obstacles to the

¹ Eicholz. Cent. f. Bact., II., ix., p. 631, 1902.

Weigmann and Zirn. Cent. f. Bact., xv., p. 463, 1894.

Marshall. Bul. 146, Mich. Exper. Sta.

proper handling of soft cheeses, since it may choke out other organisms, whose growth is desired. (See page 250.) *Oidium lactis* has no appreciable action upon milk, neither souring nor curdling it, nor making any change in its flavor in the short period before the milk would ordinarily be consumed.¹ Upon further growth, however, it gives rise to chemical decomposition, the result of which is the development of strong flavors, and



FIG. 29—OIDIUM LACTIS, FROM A SURFACE COLONY ON GELATIN

these are of significance in certain types of soft cheese and probably also in rancid butter. The organism is extremely abundant, and found practically in all dairies. It does not, however, ordinarily appear in milk or in the gelatin plates made from fresh milk, but it is almost certain to be found in old milk. It is almost always present in old butter, and may be found practically in all soft cheeses during the early process of their ripening. While, therefore, the organism has no important bearing upon milk, it is one of extreme importance in cheese making, as will be noticed later. There are several varieties of it, differing only slightly from each other.

¹ Lang and Freudenreich. Land. Jahr. d. Schw., 1893.

LIST OF MORE COMMON MILK BACTERIA

The following list gives the bacteria that are most likely to be found in milk¹:

Species almost sure to be found—

- Bact. lactis acidi* (*Strep. lactarius*).
- M. lactis varians* (*S. pyogenes aureus*).
- M. lactis albus* (*S. pyogenes albus*).
- M. lactis citreus* (*S. pyogenes citreus*).
- Bact. aerogenes*.
- B. coli communis*.
- Oidium lactis*.

Species less sure to be found, but very common—

- B. lactis fluorescens* (*B. pyocyaneus*).
- B. prodigiosus*.
- B. subtilis*.
- B. aerogenes capsulatus*.
- Yeasts*, both white and red.
- Miscellaneous molds.

¹ Bergey. Bul. 125, Pa. Dept. Agri., 1904.

For classification of Dairy Bacteria see Conn. Ann. Rep. Storrs Exper. Sta., 1906.

Düggeli. Cent. f. Bact., II., xviii., p. 37, 1907.

CHAPTER III

SOURCES OF COMMON MILK BACTERIA

Is Milk Sterile When Secreted?—The first question naturally arising is whether there are any bacteria in milk at the time it is secreted from the cow. This question has been subject to long dispute, and although many experiments have been made, there is not even yet absolute agreement. It is certain that if the udder of the cow is diseased, the milk may be contaminated, even when secreted. It has long been known that when a cow has tuberculosis in the udder her milk is practically certain to contain tuberculosis bacilli. It is equally sure that inflamed udders infect the milk with bacteria. When the udder is the seat of the inflammation it commonly produces pus, and this naturally finds its way into the milk. With the pus there are practically sure to be bacteria that are associated with the inflammatory troubles. These are usually chain forming *Streptococci*. (Fig. 3, d.) Hence, the presence of large numbers of such bacteria, especially if accompanied by numerous pus cells (see page 182), indicates a diseased condition of the udder. Such are found in animals having *mammitis* or *mastitis*. These bacteria, if in considerable numbers, are certainly detrimental to the health of the persons drinking the milk. The practical conclusion is that *no animal suffering from any udder affection* should be allowed to furnish milk for public consumption.

Whether milk at the moment of secretion from the healthy udder ever contains bacteria is not so easy to settle. Some have thought that bacteria actually come from the blood of the healthy cow, so that milk contains them when secreted. Others have been even more positive that no bacteria come from such a source, if the cow is in health. The general opinion is, however, that, at least under ordinary conditions, if the cow be in health

and her udder not injured in any way, the milk she secretes is at the moment of secretion free from bacteria.

This question is of far less importance than might seem likely, for whatever may be the condition of the milk when secreted, it is never sterile at the time it leaves the udder. Bacteria are known to be abundant in the udder. These bacteria probably come from without, entering through the teat into the milk ducts, feeding perhaps on the remains of the milk that clings to the passages between the different milkings.¹ How far into the ducts these bacteria extend is again a matter of difference of opinion.² Some have held that they are in all the ducts in the glands; others insist that the inner ducts are germ free in normal cows.³ (Fig. 30.) But these great differences of opinion are of no great practical importance, for bacteria are certainly present in the milk ducts, so that the milk, even though sterile when secreted, is practically sure to be contaminated by bacteria before it reaches the mouth of the milk duct. It is thus very difficult to draw sterile milk from the cow in spite of the greatest



FIG. 30—STRUCTURE OF COW'S UDDER

Showing ducts in which bacteria grow

¹ Ward. Bul. 178, Cornell Exper. Sta., 1898.

² Simon. Hyg. Rund., X., p. 71, 1900.

Harrison. Jour. Ap. Microscopy, V., pp. 20-29, 1903.

³ Freudenberg. Cent. f. Bact., II., x., p. 401, 1903; xiii., p. 281, 1904.

Uhlmann. Rev. Gen. d'Lait., iii., p. 163, 1904.

Russell and Hastings. Ann. Rep. Wis. Exper. Sta., p. 164, 1904.

care. By extreme precautions in special tests small quantities of sterile milk have been thus obtained; but these precautions are not practical in ordinary milking, so that the dairyman must recognize that he cannot by any means of milking obtain sterile milk.

Bacteria in the Milk Ducts.—The types of bacteria which come from the milk ducts are varied. They are mostly cocci and usually are not very numerous. Some of them liquefy gelatin and peptonize casein, while others do not; some produce small quantities of lactic acid while growing in milk, but others have no appreciable action on milk. These udder bacteria do not, as a rule, contain any representatives of the common lactic acid types, since neither *Bact. aerogenes* nor *Bact. lactis acidi* are common inhabitants of the glands or milk ducts. A very few cases have been found in which the latter organism is present in considerable abundance in the milk ducts. But all experimenters agree that this is an unusual occurrence, and that whereas it does occur in some cases, the general rule is that milk drawn directly from the milk ducts does not contain the lactic acid organisms, which in older milk are by far most common. In recent years the presence of Streptococci in milk has been sometimes regarded as rendering the milk suspicious for reasons already noted. It is very important to notice, therefore, that the freshest milk from the best of cows is practically sure to contain Streptococci coming directly from the udder. The cocci thus found appear to be similar to, if not identical with, some of the widely distributed forms that are commonly associated with inflammatory processes. (*Streptococcus pyogenes*, etc., *Staph. aureus* and *Staph. albus*, etc.) Whether they are identical with them it is difficult to say, but at all events their presence in milk cannot be a ground for condemning milk, unless it be to condemn all milk as an article of food; for otherwise nearly all fresh milk would be condemned. As a rule, the Streptococci in milk do not form

long chains, and it has been suggested that, whereas the presence of these common forms may not render milk suspicious, the presence of the long chains may do so. Upon this matter there is as yet no information.

It is quite certain that these bacteria get into the milk ducts from the outside. Some have tried to show that they may come from the tissues of the cow, being passed through the gland when the milk is secreted. But while this may be true in case the cow is suffering from some bacterial disease, such as tuberculosis or inflammation in the udder, it is practically certain that this does not occur in normal animals. If we recognize that they come from the exterior, it is natural to look for them in greatest abundance just inside of the openings of the teats. They are, indeed, found in the largest numbers just within the teats, where they enter between the successive milkings, and, in lesser numbers, further within the gland. They are in just the position to be largely washed out of the ducts by the first streams of milk drawn. This milk, called the *fore-milk*, is quite sure to contain more bacteria than milk from other portions of the milking. During the milking the bacteria may be so thoroughly washed out by the milk stream that the last milk may be quite sterile. Only certain kinds of bacteria appear to be able to live thus permanently in the milk ducts. When the experiment is tried of injecting common bacteria into the ducts, it is found that they do not live long, but completely disappear in a few days. Even the most common of dairy bacteria, *Bact. lactis acidi*, are only rarely found in the milk ducts.¹

Aseptic Milk.—The term aseptic milk has been sometimes given to milk drawn from a cow with exceptional precautions, so as to remove as far as possible bacterial contamination.² To

¹ Burr. Cent. f. Bact., II., p. 236, 1902.

² Backhaus and Appel. Ber. Landw. Inst. Univ. Königsberg, p. 73, 1900.

Willem and Minne. Rev. Gen. d'Lait., iv., p. 121, 1904.

Lux. Cent. f. Bact., II., xi., p. 195, 1903.

produce such milk the udder, flanks and under side of the cow are carefully washed; the teat washed with water or with a solution of 10 per cent. borax; sometimes a little of the borax solution is injected into the milk duct. The milker washes his hands and milking utensils, and puts on a clean milking gown. Previously a flask with a narrow neck is plugged with cotton and sterilized in the laboratory. This is taken to the milking room, and the cotton removed only at the moment of the milking. The first quarter of the milk is drawn in a separate vessel, and then the mouth of the flask is placed close to the teat, and the milk is drawn directly into it, after which it is immediately closed again with the cotton plug. In this way the chance of external contamination is practically eliminated, and the milk which is obtained contains only the bacteria from the udder itself. Such aseptic milk contains much fewer bacteria than ordinary milk, but even with these precautions sterile milk is not obtainable. It usually contains, however, only the harmless cocci, which have little effect upon it, and it may be kept for a long time without souring or showing other changes due to milk bacteria. Such a method of milking is, of course, impractical. A somewhat close approximation to it is obtained by modern milking machines, which will be referred to on a later page.

EXTERNAL CONTAMINATION

Most of the bacteria in ordinary milk come not from the udder, but from external sources. These sources are several, and may be considered in succession.

The Cow.—One of the chief sources of bacteria is the cow herself. Her skin, even when kept in fairly good condition, is never very clean and will always hold more or less dirt and dust laden with bacteria. The cow in many poorly kept dairies is, as is well known, rarely, if ever, cleaned: her flanks, tail and skin become covered with a coating of manure, until



FIG. 31—FILTHY CONDITION OF COW'S HINDQUARTERS TOO FREQUENT ON DAIRY FARMS

the amount of filth attached thus to the animal is surprisingly great. All of this filth is laden with bacteria in large numbers. (Figs. 31 and 32.) It is impossible for the cow to move without more or less of it falling from her body, and during the whole process of the milking, by the movements of the flanks,

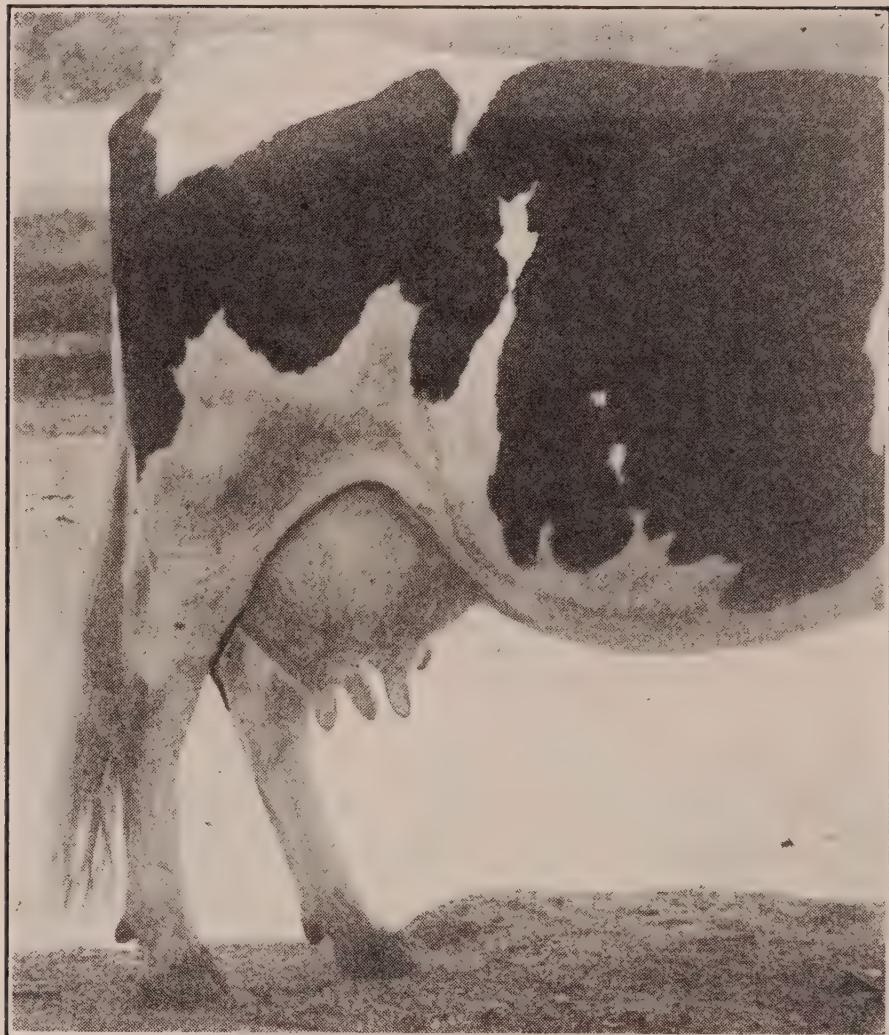


FIG. 32—PROPER CONDITION FOR COW'S HINDQUARTERS

the rubbing of the skin by the milker, and by the switching of the tail, there is constantly being shed a shower of such particles from her body. This continues during the whole milking; and the milk pails, which are generally widely opened, are calculated to receive as large an amount of it as possible. The filth that thus finds its way into the milk pail consists of almost every conceivable kind of material. Besides excrement there are grass, straws, insect wings, hairs and almost every other kind of small particles. (Fig. 33.) The nature of this dirt and its

amount depend upon the condition of the cow. There will be less on cows during the pasturing season than during their life in the barn in the winter. It will vary much with different foods. Dry foods, like hay, appear to furnish more bacteria in the manure than moist foods, and the milk subsequently receives them. It frequently happens that a certain food fed to the cows injuriously affects the keeping of the milk because the bacteria, which the food furnishes, find their way into the milk from the filth on the cow; for example: the feeding of turnips.

The Air.—In earlier studies of bacteriology, it was thought that the greatest source of bacterial contamination was the air. More careful study of later years has produced considerable change in this belief. Fresh air out-of-doors does not contain many bacteria, and if milking could take place in the open, free air, this source of contamination would be almost excluded. In a close barn, however, conditions are very different. The motions of the crowded cattle are dislodging bacteria from their skins, which float for some time in the air. Hay, dirt, cobwebs and other dry dust-producing materials are allowed to accumulate. Dirty straw bedding is another source of air contamination. (Fig. 34.) The general manner of feeding the animals is even a larger source of contamination. If dry hay, or other dry food, is thrown down in front of the cattle, a large



FIG. 33—NUMEROUS COLONIES OF BACTERIA GROWING FROM COW'S HAIR PLACED IN GELATIN

¹ Backhaus. Milchzg., p. 357, 1897.

amount of dust will arise and spread through the air of the stable. Such dust is crowded with bacteria, many of which are alive and ready to grow. The common practice of keeping the cattle in the same room where they are milked is thus very productive of a large source of air bacterial contamination.¹ The number of bacteria coming thus from the air varies im-

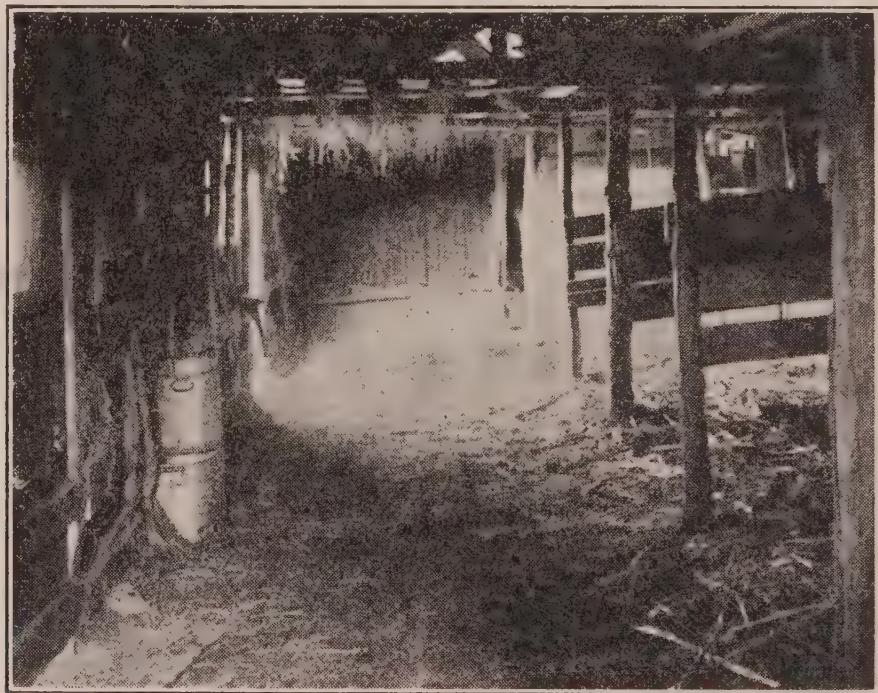


FIG. 34—POORLY-KEPT COW BARN, SURE TO GIVE HIGH BACTERIAL CONTACT TO MILK

mensely with the conditions of the stable. It is largest during and immediately after the feeding, especially when cows are fed upon dry food. The longer the time elapses after feeding, and the longer time the bacteria have to settle to the floor, the clearer the air becomes. But in the crowded conditions of the stables and the constant motion of the animals and their attendants, the bacteria never settle entirely out of the air and will always be a source

¹ Barthel. Rev. Gen. d'Lait, V., p. 505, 1902.

of contamination. If milking takes place immediately after feeding, the number of bacteria from this source is very high. These facts are easily demonstrated by simple tests. The air of the dairy and the out-of-door air to which the milk may be exposed are also sources of some contamination, but these are usually slight. One curious instance has been recorded of milk being filled with troublesome organisms from the open cans standing under trees on the leaves of which the organism was growing. The bacteria derived from the air are, as a rule, harmless forms. *Bact. lactis acidi* is undoubtedly in many cases derived from this source. We do not positively know the original source of this germ, although Barthel has found it upon the leaves of growing plants, whence it might easily get into hay.¹ But we do know that it gets into the milk from the dust and the air of the milking stable, and from the dirt attached to the skin of the cows, from faeces and indeed from almost all objects in the barn. *Bact. aerogenes* probably also comes from the air. Other bacteria from this source are representatives of the liquefying type and tend to produce liquefaction. Many neutral bacteria are also found.

Milk Vessels.—Perhaps the most prolific source of milk contamination, so far as numbers are concerned, is found in the bacteria which remain alive in the milk utensils between one milking and the next. Bacteria are not usually killed by short drying. After each milking the milking pails and other utensils are commonly washed, sometimes, indeed, thoroughly washed with hot water and soap; but no such washing is sufficient to destroy the bacteria which may be in the pails. No simple washing will make a milk pail *bacteriologically* clean. After any ordinary washing there will be left clinging to such utensils many bacteria which are ready to develop as soon as fresh milk is added to furnish them with proper food and proper condi-

¹ Barthel. Rev. Gen. d'Lait, V., p. 223, 1906.
Burri. Cent. f. Bact., II., x., p. 756, 1903.

tions. Every different vessel or utensil with which the milk comes in contact, on its way from the cow to actual consumption, adds its quota of bacteria.

Probably the various vessels and utensils supply the largest source of milk bacteria.¹ The great difficulty of keeping these clean leaves them almost filled with bacteria. As the milk passes from the cow through one utensil to another it gathers more and more bacteria. For example, in a rather badly kept dairy the following figures were found by Bergy:

Number of bacteria in milk from udder	400
Number of bacteria in milk in bucket	800
Number of bacteria after passing strainer	60,000
Number of bacteria after passing cooler	34,000
Number of bacteria in tank below cooler	173,000
Number of bacteria after passing bottler	84,000

While these numbers do not regularly increase they do show that there is a growing contamination from the various utensils with which the milk comes in contact. The irregularities in the results are doubtless due to the difficulty of getting average samples, always a great difficulty in such analyses.

The Milker.—Of late years it has become more evident that the bacteria from the milker or other persons in the dairy are among the most serious. This is not so much because of the number of the bacteria that may enter the milk from this source but of their types. In ordinary dairies the milker rarely makes any special toilet before milking, and is liable to perform this task in old, soiled clothes, with no special attempt at cleaning his hands or face. Under these circumstances, while he is not so great a source of bacteria as the cow, some of these organisms are sure to fall from his hands or clothing into the milk vessels. The number of bacteria from such a source is, probably,

¹ Erf. and Meleck. Bul. No. 131, Kan. Exper. Sta. 1905.

not great and does not add materially to the bacterial content. But in one respect these bacteria assume a more important significance. The bacteria which produce diseases in one animal do not necessarily produce diseases in other animals. Those which produce diseases in cattle, with some exceptions (tuberculosis), do not usually have the same effect on man. But it is evident that any disease germs that may be present in one man are just the kind that can develop in any other human being. Therefore, the bacteria contamination from human sources is more dangerous to other human beings than any infection from animals. For this reason the bacteria which enter the milk from the milker are liable to be much more dangerous than those which come from any other source. This we shall consider in the next chapter.

SOURCES OF COMMON MILK BACTERIA

While at present no complete statement can be made as to the sources of all the milk bacteria, a few facts concerning some of the more common are of value.

The common Streptococci and other coccus forms appear to come mostly from the udder directly. In some cases this may indicate a diseased condition of the udder, but even from the healthiest cow the milk will be sure to show these types at the outset.

Bact. lactis acidi is derived, in most cases, not from the udder but from the dust of the air, or the hair of the cows, and possibly in some cases from the faeces. But beyond doubt the largest source of these germs in the ordinary dairy is the milk vessels. This species grows abundantly in milk, and will always be left in large numbers in the milk vessels, even after thorough washing. Hence, milk vessels are its chief source. Its original source has not been so easy to trace. It is sometimes found in the feed of the cattle, and Barthel has found it upon the

leaves of plants, where he thinks it multiplies naturally. Whether this may be regarded as its natural home cannot yet be stated. It is suggestive to note, however, that when found in fresh milk it does not appear to be in a vigorous condition, and grows much less rapidly than it will after it has been developing in milk for a number of hours. This suggests that whatever be its immediate source in the dairy it does not come from a location especially well adapted to its life.

Bact. aerogenes and other *gas-producing organisms* are also from sources external to the cow. They practically never come from the cow's udder, but chiefly from the dirt of the cow or from the faeces, and they may, also, be derived from the air or other external filth contamination. Sometimes they, too, will be found in incompletely sterilized milk vessels. The practical conclusion to be drawn is that the dairyman's method of avoiding the troubles arising from gas-producing organisms is the extra care taken to prevent external contamination of the milk, or extra thoroughness in washing vessels.

B. coli communis.—This organism, so far as we know, comes wholly from faecal contamination. It is quite common in milk, as would be expected from the well-known fact that it is a constant inhabitant of the intestine, and that almost all milk is contaminated with manure. Its presence in milk would prove a very serious contamination, if the faecal contamination were from human beings, but inasmuch as it comes from cows the contamination is less serious than it would be from a human source.

The other types of bacteria found in milk need not especially detain us here. The udder itself furnishes merely harmless germs, at least in cases where the udder is free from all disease. The kinds of bacteria which produce trouble in milk, *slimy milk*, *bitter milk*, etc., come from such a miscellaneous variety of places that no definite sources can be named. In cases of trouble each must be traced to its own source, which

is sometimes the udder, sometimes the food, sometimes the faeces, sometimes the water, sometimes infected cans from a creamery or cheese factory, etc. The dairyman must simply use his own ingenuity in detecting the source of the trouble in each individual case.

SIGNIFICANCE OF DIRT IN MILK

From the facts above outlined it is evident that the dirt which gets into milk is a very important source of organisms which are likely to produce trouble, since they may injure the milk or butter, and may produce even more trouble in cheese-making. The presence of dirt means infection with bacteria and, in many cases, with types of bacteria that are emphatically mischievous in the dairy; it always means filthy dairy conditions. The recognition of this fact has led to attempts to estimate the amount of dirt present in milk, as one of the means of determining its quality. Milk which contains a large amount of dirt must be far inferior in quality to a clean milk, since it will contain more bacteria. Clean milk will keep better, and it will be less liable to contain disease germs. Several methods of estimating the amount of dirt in milk have been devised, none of which are very accurate or very satisfactory. These are described in experiment 46.

The methods there given will give the dry weight of the solid, insoluble particles of dirt which get into the milk, and which may be filtered out. But they do not give any estimation of such particles as are too small to separate out by sedimenting or centrifugalizing, and they give no indication of the portion of the dirt that may have been dissolved in the milk. The absolute amount of dirt found is of no special significance, but if numerous estimations are made by the same individual on a series of milk samples, the relative amounts will indicate the relative cleanliness of the different samples. Estimates made

by different observers do not agree very closely because of slight differences in technique. Figure 35 shows the amount of dirt collected under the udder of a cow during milking, both with, *b*, and without, *a*, previous washing of the udder. The amount is certainly sufficient to emphasize the importance of this source of bacterial contamination.

The determination of the amount of dirt in milk certainly gives a gauge as to the care exercised in its production. But whether such dirt determination can be of any value in the

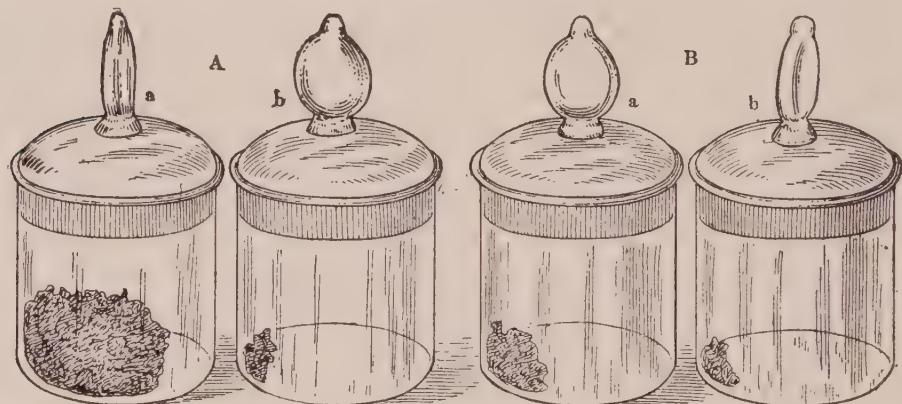


FIG. 35—RELATIVE AMOUNT OF DIRT FALLING FROM THE UDDER DURING MILKING, UNDER DIFFERENT CONDITIONS

A. Udder muddy. *B.* Udder slightly soiled. One-half natural size. (Fraser)

municipal testing of milk is somewhat questionable. It is not yet possible to give any standard as to the amount of dirt that may be allowed in passable milk, and there seems to be no reason for believing that the dirt determination will be adopted as one of the methods of municipal examination of market milk. If milk is allowed to stand for several hours in a glass jar until the dirt settles at the bottom, an examination with the naked eye will give the relative amount of dirt, and the results will probably be as valuable as the longer laboratory methods of determining the quantity, especially when it is remembered that the amount of dirt thus obtained varies quite widely with different experimenters and different methods. Special dirt filters

have been devised by which the amount of dirt can be quickly determined. These are intended to be used by milk inspectors in their ordinary routine examination of market milk. They have not as yet come into common use.

A brief summary of the facts of this chapter may be useful. The bacteria that get into the milk from the udder are mostly cocci. Most of these are harmless, and they will always be found. Some appear to be identical with forms that are associated with various forms of inflammation, and if these be abundant they may make the milk unwholesome. None of them have any noticeable effect on the milk. An unusual number of Streptococci is usually accompanied with pus cells, and injures the milk. The bacteria that get into the milk from other sources are those common in air, soil and water, most of which are harmless. Among these are two types of lactic acid bacteria, various forms of putrefactive organisms and large numbers of miscellaneous species. From these sources, too, come the bacteria that occasionally produce trouble, such as those causing slimy milk, blue milk, tainted milk, sweet curdling, etc. Some of these produce great losses in cheese-making by causing undesirable flavors. The putrefactive bacteria are always undesirable but fairly common. From the milker or dairyman may come certain dangerous disease germs.

Nearly all dairymen, to-day, have come to recognize the desirability of keeping the number of bacteria as small as possible in milk, and are looking for methods by which this can be done. In order to consider intelligently methods by which a dairy can accomplish this it will be necessary to learn certain important facts concerning bacteria growth. The practical application of the facts just given will therefore be reserved for a later chapter.

CHAPTER IV

THE GROWTH OF BACTERIA IN MILK

THE original bacterial contamination of milk accounts for the bacteria that may be found in fresh milk, but gives no idea as to what may be found at any subsequent time. The rapid growth of those which get in and the great differences in rapidity of growth of different species are such that the bacterial content of any sample is undergoing constant changes. To understand dairy problems we must study the *growth* as well as the source of milk bacteria.

Bacteria in Normal Milk.—The number of bacteria in fresh milk varies widely. It may be as low as 100 per c.c. in some of the best dairies, or it may be as high as half a million, or even higher, in the poorer type of dairy. Absolutely no average can be given that has any meaning, for, as will be pointed out in a later chapter, the number will depend upon the methods used in the dairy. The kinds of bacteria present in different samples of fresh milk will also vary widely. If, however, we take the milk of an average dairy kept in fairly good condition, we should find the bacteria in fresh milk somewhat as follows:

There will be very few of the *Bact. lactis acidi* type. These are frequently so few that they cannot be detected by the common methods of analysis. There may be one or two per cent., or sometimes more, but they are always in small numbers, rarely as high as 10 per cent. Their number is largely dependent upon the cleanliness of the milk vessels. Gas-producing acid organisms are also liable to be present; but these, too, are few in numbers in good milk, and their presence in any considerable numbers is unfortunate, because they are liable later to produce

trouble in the milk. The largest part of the bacteria will be *cocci*, some of which liquefy gelatin and belong to the peptonizing group, and some of which do not. These organisms come directly from the udder and can practically never be avoided. Their presence in milk is normal, even in the best dairy conditions, and there is no reason for regarding them as injuring the milk, for most of them have no action upon it and are perfectly harmless to man. In addition to these there will be a varying number of *miscellaneous bacteria*, liquefiers and non-liquefiers. Their number and types will depend upon the extent of the contamination of milk with manure, dust and other sources of bacteria. These miscellaneous bacteria are the ones most likely to produce trouble.

EFFECT OF TEMPERATURE ON NUMBERS

The number of bacteria in milk at any subsequent period is more largely dependent upon the temperature and age than upon the original contamination.¹ Milk when fresh from the dairy, even from the slovenly, filthy dairy, will rarely contain more than half a million or so bacteria per c.c., and usually a much smaller number. By the time the milk is one or two days old the numbers may amount to 50,000,000, to 100,000,000, or even more. Milk from an ordinary cleanly, carefully kept dairy may have no more than from 10,000 to 50,000 per c.c. at the outset. But this milk, also, in the course of one or two days may have numbers as high as 100,000,000. The number of bacteria present at any time is, therefore, more dependent upon the age of the milk and the method of keeping it than upon the original amount of contamination. Milk which originally contained large numbers of bacteria, if kept cold, will in 24 hours show a much lower bacteria count than milk which originally had small numbers but was kept under warmer conditions. This

¹ Conn. Bul. 26, Storrs. Exper. Sta., 1903.

should always be considered in drawing any conclusion as to the numbers of bacteria in milk. In ordinary municipal testing of milk this feature is too frequently lost sight of, or, at least, not properly considered.

A single illustration will suffice to show the effect of the different temperatures upon the numbers of bacteria. A sample of milk was examined for bacteria when fresh, and was found to contain 6,525 per c.c. It was then divided into two lots, one preserved at 50° and the other at 70°.

GROWTH OF BACTERIA AT 50° AND 70°

Fresh milk	6,525 per c.c.
25 hours old at 50°	6,425 " "
25 hours old at 70°	6,275,000 " "

The difference in this case is very striking. In these two samples of the same milk one showed no more bacteria at the end of 24 hours than at the beginning, while the other had increased 1,000 fold. It is not common to find quite such a marked difference, but in all cases the difference in the number of bacteria in two samples thus tested is very great. It is important to note that these two samples of the same milk would receive very different verdicts from any municipal examination. One would be passed as an exceptionally good milk, and the other condemned as a very bad milk. But if the bacteria in the fresh milk were the common harmless species, liable to be found in milk, the sample which at the end of 24 hours contained 6,000,000 would be just as wholesome as the one that contained 6,500. In other words, the ordinary examination of milk, which condemns milk containing more than a certain number, fails to reach the desired end. It does not distinguish between good milk, in which the harmless lactic bacteria have become quite abundant, because of a few extra degrees of temperature, and unwholesome samples of milk, in which the num-

bers of bacteria are due to the fact that the milk contains a large amount of filth, with its special and injurious types of

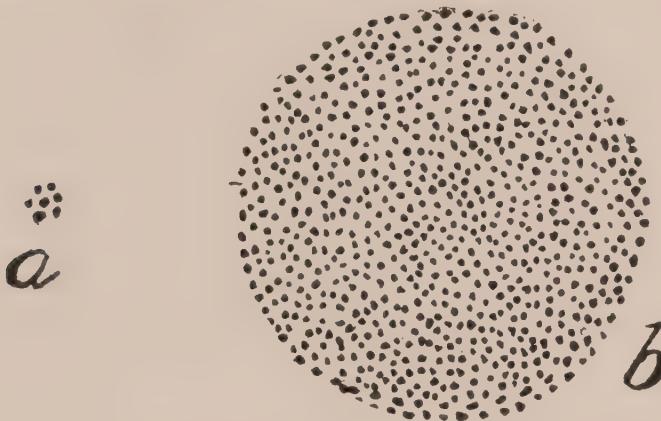


FIG. 36—GROWTH OF BACTERIA AT DIFFERENT TEMPERATURES DURING 24 HOURS

Each dot representing a single individual
 a. At 50° : : : 7 bacteria
 b. At 70° : : : 700 bacteria

bacteria. The bearing of this upon the question of municipal examination will be considered later.

The rapidity of growth of common bacteria at different temperatures is graphically shown by the accompanying figure 36.

THE GROWTH OF DIFFERENT SPECIES

When drawn from the cow milk is at a temperature that especially favors the development of most ordinary species of bacteria. While different species differ somewhat as to the temperature at which they grow best, a majority of them can multiply most rapidly at about body heat, between 90° and 100° . Some species, however, find a lower temperature especially favorable. Hence the *kinds* as well as the numbers of bacteria in milk are largely affected by the temperature.¹

At 33° .—If milk is frozen, bacteria do not grow at all. If it is only a degree or two above freezing, they grow very slowly,

¹ Conn and Esten. Ann. Rep. Storrs. Exper. Sta., 1901.

Conn and Esten. Rev. Gen. d'Lait., i., p. 121, 1901.

so slowly indeed that for several days there is practically no increase in numbers. But even if milk is kept only one or two degrees above freezing, the bacteria will increase, and will in the course of time—in two or three weeks—be found to be extremely abundant. It is important to note that the bacteria which are found at these low temperatures do not produce the ordinary changes in milk. The milk does not sour or curdle and, as a rule, there is no visible sign in such milk that the bacteria have become abundant. Such milk may have a somewhat oldish taste, but, as it is neither sour nor curdled, would usually be pronounced sweet and would naturally be used with perfect freedom. The milk, however, may contain bacteria in great numbers, and may become harmful because of them.

At 40°.—If the milk is warmed to about 40°, nearly the same statement can be made, excepting that the bacteria tend to grow a trifle faster and will become abundant somewhat earlier. It cannot be emphasized too strongly that milk or cream retained at these low temperatures, while it will remain sweet for many days, and even for some weeks, cannot after the first few days be looked upon as wholesome, since the bacteria have been growing, even though slowly, and may be present in enormous numbers. It may be possible that some cases of milk poisoning, especially such as come from ice cream, may be attributed to this cause. (See page 109.) The emphatic lesson to be drawn is that no normal milk that has been kept for many days is safe, even though it still remains sweet.

At 50°.—If the temperature is raised to 50°, the bacteria develop more rapidly, although very much more slowly than at higher temperatures. At this temperature the species which grow are commonly not lactic bacteria, but a large variety of *miscellaneous forms*. This is a matter of some importance, since while lactic bacteria are harmless and in some respects useful, the miscellaneous types which develop at 50°, though many of them are harmless, are more likely to contain species

which give ptomaine poisoning and possibly summer complaint and cholera infantum.

At 60°.—If the milk is warmed to from 60° to 70°, the growth of bacteria is very much more rapid. It is extremely important to notice the type of bacteria specially favored by this temperature is the normal, harmless, *useful lactic acid bacteria* and *Bact. lactis acidi*. If milk is kept constantly at 60°, the lactic acid bacteria multiply far more rapidly than any other species. Examinations of milk at intervals of an hour or two show a constantly increasing proportion of this type. At the outset the number of lactic acid bacteria is comparatively small, frequently less than 1 per cent. and seldom as high as 10 per cent.; but they constantly increase hour after hour, and when the milk is 24 hours old, the lactic acid bacteria are apt to comprise 50 per cent. of the whole. In another day, just about the time the milk is ready to sour, the proportion of this type is commonly over 95 per cent. and sometimes 100 per cent. In other words, if milk is preserved in a temperature from 60° to 70°, the lactic acid bacteria far outrun other species and soon take their place. The acid which they develop seems to be injurious to the other species, and these rapidly disappear as the lactic organisms increase in numbers. As a rule, therefore, *milk which sours at a temperature of 70° or thereabouts will be found to contain nearly a pure culture of the common lactic acid type*, the other organisms that were so abundant at the outset having disappeared.

For this reason we understand why lactic acid bacteria are regarded as a dairy bacteria par excellence. Although this species is not present in fresh milk in great numbers and constitutes only a small proportion of the whole, yet by the time the milk is 24 hours old, at ordinary temperatures, they have so outnumbered the others that they constitute the larger proportion of the bacteria present.

At 80° to 100°.—If the milk is kept at temperatures from 80° to 100°, the results are far more variable. The bacteria grow very much more rapidly, but we cannot tell so surely what species will develop in greatest abundance so as to become predominant. In some cases here also the *Bact. lactis acidi* assumes predominance and sours the milk; but in a great many other cases, apparently the majority, some of the other types assume the upper hand in milk. It frequently happens that at these higher temperatures the gas-producing bacteria, *Bact. aerogenes*, become especially abundant, and by the time the milk has soured it will contain large proportions of these organisms that are so mischievous in various dairy products.

For this reason it is extremely important that milk to be used for butter-making, and especially for cheese-making, should be kept at temperatures below 80°, and after the curdling by rennet should be cooled to below 80°. The presence of the gas-producing organisms is sure to produce mischief, whereas the proper growth of the other lactic bacteria is a necessary accompaniment to a good butter or cheese. From the facts here outlined it is evident that dairymen should endeavor to get milk quickly to a temperature below 70° in order to stimulate the development of the proper lactic acid bacteria. It should be remembered, however, that if the starter of the proper organisms be added to milk or cream so that this organism is present in special abundance, a higher temperature of 80° may frequently be safely used to hasten the souring of the milk. Under these circumstances, since the desirable organisms are added in great abundance, they succeed in producing their action on milk in spite of gas-producing organisms or other bacteria that may be present in smaller quantities. If, however, ordinary milk or cream, without a starter, be ripened at a temperature of 80°, or thereabouts, the results will be very uncertain, and in some cases gas production will give rise to trouble and serious loss.

PHASES OF BACTERIA GROWTH IN MILK

Germicidal Phase.—For some time after milk has been drawn from the cow bacteria fail to develop; indeed, many tests have shown that for the first few hours there is an actual reduction in the numbers of bacteria.¹ The exact reason for this *reduction* has been the subject of some discussion, but the different ways of explaining it need not occupy our attention. The general feeling has been that there is present in milk some substance which is inimical to some kinds of bacteria and actually destroys them. For this reason this period has been called the *germicidal* phase. It lasts for a varying time, depending largely upon temperature. It commonly continues only a few hours, sometimes not more than two. At other times it may last for several hours, especially if the milk is kept cold. (See experiment on page 297.) The practical value of this fact is simply that for a period of a few hours after milk is drawn the bacteria do not develop, and the handler of the milk has, thus, a short period in which he need not fear much bacteria growth, and during which he can bring the milk into conditions for further preservation.

First Period of Growth.—Following the germicidal phase the bacteria grow with a rapidity depending, as we have seen, on temperature.² For a number of hours there is an increase of most species. Most of the kinds of bacteria present in the milk find it a favorable medium, and multiply with varying degrees of rapidity according to the nature of the species. But some multiply more rapidly than others, so that the proportion of the different types will constantly change as the hours pass. For a period of 12 hours, at ordinary temperatures, there is

¹ Hunziker. Bul. 197, Cornell Agri. Exper. Sta., 1901.

Koning. Milch. Zent., I., p. 99, 1905.

Mayer. Rev. Gen. d'Lait, II., p. 381, 1903.

² Koning. Rev. Gen. d'Lait, 1904.

Gissier and Gasching. Ann. d. l'Inst. Past., xvii., p. 540, 1903.

a fairly regular increase of nearly all species, although the common lactic bacterium grows more rapidly than any other. The length of time of this period of increase depends upon the temperature of the milk. If kept cold, there may be a day, or even two days, during which time there will be shown a general increase in the numbers of nearly all kinds of bacteria.

Second Phase.—After the numbers of bacteria have become fairly large, 10,000,000 to 20,000,000 per c.c., it is found that the *Bact. lactis acidi* has gained the upper hand of the others.¹ This species, present in small numbers at the outset, has increased in proportion until at the close of the first period there may be from 20 to 40 per cent. of them. During the second period, which now follows, they rapidly increase until they finally come to be nearly 100 per cent. This type of organism has, in other words, almost or completely replaced the numerous species found at the outset. This second phase ends with the souring and the curdling of the milk.

Third Phase.—After souring and curdling, the bacteria in the milk cease growing, the amount of acidity being so high that they cannot thrive. Many of the bacteria die, and there is a fairly rapid reduction in numbers until, after a few days, they are once more very few. The milk may now remain for a long time in this condition without further bacteria growth. Later, however, there are sure to develop some conditions which reduce the acidity. This third phase, however, does not affect the milk handler, but chiefly the cheese producer; for it is only in milk products that have been kept many days, or several weeks, that the later changes occur. These later changes are variable, but, in general, they consist of a growth of molds over the surface of the milk or cheese, which reduces the acidity to a neutral, or slightly alkaline, condition. After the acid has disappeared, bacteria will again begin to grow. The bacteria which develop in the third phase are not lactic germs,

¹ Bienstock. Arch. f. Hyg., xxxix., p. 390, 1901.

but belong more commonly to the type which liquefies gelatin, digests casien and produces general putrefaction and decomposition. In the end, unless it dries, they cause the putrefaction and decay of the product, a change that would have occurred in the milk earlier if it had not been for the protecting action of the lactic bacteria. These further changes, however, need not be considered at this point.

The phases above described occur normally in milk that is preserved at a temperature not above 70° . If the temperature is higher, the changes will be quite different. The different stages will not only follow each other more rapidly, but the souring of the milk will be produced by the growth of the different kinds of bacteria, and the resulting material will be of a totally different nature. It may be full of gas bubbles, it may have vile odors, but, in most cases, it will be a product which dairymen will recognize at once as abnormal and undesirable.

While the phases above outlined may be taken as illustrating the general changes in milk, they can only be looked upon as representing an average. Different samples will differ very much. Sometimes the milk will contain bacteria more vigorous than the lactic acid type, and these will gain the upper hand, as, for example, when the slimy bacteria are present. Moreover, the various bacteria in a sample of milk have influence upon each other. Sometimes one species will hasten the growth of another species, as shown by Marshall,¹ and sometimes one will delay the growth of others. The phenomena are so widely varied with different samples of milk that any description will be only approximate. The course as given above represents the usual series of events when milk contains the normal bacteria and is kept at about 60° to 70° .

¹ Marshall. Cent. f. Bact., II., p. 400, 1905.

Bouska. Rev. Gen. d'Lait, III., p. 1, 1903.

PROTECTIVE ACTION OF THE LACTIC ACID BACTERIA

The rôle which the lactic bacteria play in milk is one of considerable importance, since they protect the milk from putrefaction.¹ Most proteid materials readily putrefy from the action of bacteria. Why does not milk undergo the same change? The other bacteria which are liable to be found here, especially the liquefying type, if left to develop unhindered, would produce a series of decomposition changes in the milk, as they do in meat or eggs, resulting in a variety of bad tastes and odors, ending in putrefaction, and in some cases would produce poisons, which would be extremely injurious. But ordinarily milk will not putrefy. The rapid development of the lactic acid bacteria prevents the growth of these miscellaneous organisms. As a result, although it contains a great quantity of easily putrefiable proteid, it is prevented from undergoing this putrefaction by the development of the lactic acid bacteria. One practical side of this question relates to the pasteurizing of milk. As we shall notice later, the ordinary pasteurizing of milk destroys a large number and sometimes all of the lactic bacteria, but leaves in an active condition many kinds of bacteria, especially those producing spores. It frequently happens, therefore, that in pasteurized milk there are no lactic acid bacteria present whose growth can subsequently prevent the milk from putrefaction by means of the other bacteria. Pasteurized milk will ordinarily not sour and seems to keep good for a considerable time; but it inevitably undergoes certain types of decomposition, and the products which appear after the bacteria have begun to grow are very much worse than those in sour milk. Pasteurizing, therefore, deprives the milk of the protecting action of acid bacteria, so that after the other bacteria have a chance to grow, as they are sure to do in a few days,

¹ Bouska. Land. Jahr. der Schweiz, 1903.

the milk becomes very vile and unfit for use. These facts are of much more importance in cheese-making than anywhere else. When the milk is used for drinking it is almost sure to be consumed before this protective action becomes noticeable; but cheese, the casein of milk, must be kept many weeks for ripening. To a less extent the facts are true of the ripening of cream in butter-making. We thus see the reason why lactic bacteria are necessary in butter and cheese-making, and why starters have become so widely used. It has even been seriously suggested that milk to be used for drinking should be inoculated with lactic bacteria to insure their presence and aid in the exclusion of other species. Here, too, we find an explanation of the fact that sour milk and butter milk are frequently recommended for drinking, these products being practically sure to contain large numbers of the lactic acid bacteria.

From all of these facts it will be evident that the question of number in the bacterial content of any sample of milk is a very complicated one. The original contamination is only one factor and a small one. The age of the milk, the temperature at which it is kept, the relative abundance of the different species at the outset, the extent to which the different species are favored by the temperature, the influence of one species upon another,—all these, and other factors as well, combine to determine the bacterial content of any sample of milk. It is manifestly impossible to make any general statements as to what numbers or kinds of bacteria should be found in any sample of milk.

CHAPTER V

DISEASE GERMS IN MILK

THERE is a wide-spread feeling that the bacteria in milk are closely associated with the distribution of diseases.¹ This idea is undoubtedly well founded, but in the minds of most people it is too indefinite to have much meaning, and its very indefiniteness gives rise to erroneous impressions. The simple fact that milk contains bacteria does not render it dangerous to drink, nor that it contains large numbers of bacteria. It has been known for many years that sour milk is a perfectly healthful product, and physicians, to-day, frequently recommend sour milk or buttermilk as a diet for invalids or infants.² Now, for reasons that we have already noticed, it is perfectly clear that buttermilk and sour milk will contain bacteria in enormous numbers. Buttermilk may frequently contain as many as 500,000,000 bacteria per c.c., and sour milk will contain them, sometimes, in equal quantity. The fact that these beverages, with such enormous numbers of bacteria, are perfectly healthful and wholesome, is a clear indication that the simple presence of bacteria in milk is not the fact which renders it suspicious, or in any degree less wholesome.³ Indeed, buttermilk is frequently recommended as a diet *because* it contains such large numbers of bacteria. It clearly follows that the impression which many people have that large numbers of bacteria render milk dangerous is a mistaken one. Any attempt to determine the healthfulness or the harmfulness of milk based upon the number of bacteria which it contains is, therefore, fallacious.

¹ Busey and Kober. Rep. Health Off. of Dist. Col., 1895.

² De Mattos. Jahrb. f. Kinderheilk., LV., p. 1

Salge. Jahrb. f. Kinderheilk., LV., p. 157.

³ Bienstock. Ann. l'Inst. Past., XIV., p. 750, 1900.

If the simple presence of bacteria in great numbers does not make milk dangerous, it must be that certain kinds of bacteria are harmful, while others are harmless. To understand the relation of milk bacteria to health, therefore, it is necessary to examine more closely the question of the kinds of disease germs liable to be in milk.¹ The accumulated information of the last 20 years has shown us that four specific diseases are commonly thus distributed, together with a fifth type of disease which is less definite. The specific diseases are *tuberculosis*, *typhoid fever*, *scarlet fever* and *diphtheria*, and the less definite type includes the diarrheal troubles known commonly by the name of *cholera infantum*, *summer complaint*, *infantile diarrhea*, etc. These are all characterized by diarrheal disturbances, but with such varying symptoms that they are not recognized by physicians as sharply defined diseases, and they are moreover produced by more than one kind of a germ.

DISEASES DIRECT FROM THE COW

Diseases that come directly from the cow are clearly more difficult to avoid than those which come from external sources. Fortunately, however, the diseases that attack cows do not usually attack man, and, with one exception, are not positively known to be distributed by milk except in the rarest of instances. The one disease thus distributed is tuberculosis.

TUBERCULOSIS

Extent of Tuberculosis.—The very great publicity that has been given in recent years to problems associated with tuberculosis in cattle renders it unnecessary here to do more than summarize the chief conclusions. It is, to-day, thoroughly recognized that this disease is one of the most serious menaces

¹ Klein. Jour. of Hyg., i., p. 78, 1901.

Ernst. The Infectiousness of Milk, Boston, 1895.

Lee. Ann. Pub. Health Assn., 1898.

Wilckens. Zeit. f. Hyg., xxvii., p. 264, 1898.

to the dairy industry. It is known to be present and increasing in our dairy herds.¹ It is known that in the more thickly settled parts of this country, as well as in Europe, the percentage of the disease among cattle is quite large, although it is difficult to give exact percentages. In Denmark and Germany 40 to 50 per cent. of the cattle are affected. In cold climates where the cattle are kept housed much of the time it is more common than in warm countries where they remain out of doors. In America it does not yet appear to be as common as in Europe; but still in the dairy districts of this country it is also very common. It is apt to run through a whole herd after it once gets a foot-hold. Sometimes practically every animal in a herd will have the disease, while in another herd none will be found. With such wide differences averages mean little. The disease is frequently introduced into a herd by the purchase of stock that appears to be healthy but is really infected with the disease. Hence, highly bred stock is most likely to be found infected.

Statistics have also shown that the disease has been rapidly increasing among cattle in the last 25 years. Such statistics, it is true, are open to some criticism. 25 or even 15 years ago the importance of tuberculosis in cattle was not recognized, and the method of detecting the presence of the disease was uncertain; careful records as to the percentage of tuberculosis among cattle were then scanty and unreliable. It is only about 15 years ago that any very strenuous attempt was made to determine the percentage of tuberculosis among cattle. As years have passed the importance of the matter has drawn more attention to it, and statistics have been collected with more and more thoroughness and completeness. Such in-

¹ Salmon. 20th Ann. Rep. Bu. An. Ind., p. 89, 1903.

Salmon. Bul. 53, Bu. An. Ind., 1904.

Salmon. Bul. 38, Bu. An. Ind., 1906.

Salmon. Bul. 33, Bu. An. Ind., 1901.

creasing completeness of inspection and examination would inevitably seem to show that the disease is on the increase. For this reason, and for others that we need not mention, the statistics are open to criticism. In spite of the criticism, however, no one questions the general belief that this disease has been on the increase among cattle, though how rapidly we do not know. It is now agreed by all that tuberculosis is, perhaps, the most serious problem that the dairyman has to face and one that threatens to undermine the whole industry.¹

Tubercle Bacilli in Milk.—So far as concerns our problems, however, it is the relation of this tuberculosis to milk and the distribution of the disease from animals to man that interests us. The most important facts may be summarized, as follows:

Animals suffering from tuberculosis may, under some conditions, produce milk that contains tubercle bacilli. This has been proved by studying the milk from such animals by two methods: 1. The inoculation of such milk into susceptible animals, like guinea-pigs, results, in many cases, in the guinea-pigs rapidly acquiring tuberculosis.² 2. A microscopic study of such milk has demonstrated the presence of tuberculosis bacilli. (Fig. 37.) Although the microscopic examination of milk may



FIG. 37—TUBERCULOSIS BACILLI
(LEHMANN & NEUMANN)

¹ Markl. Milchzg., p. 232, 1901.

Beck. Zeit. f. Fleisch u. Milch Hyg., xi., p. 245, 1901.

² Mohler. Bul. 44, Bu. An. Ind., 1903.

Schroeder and Cotton. Bul. 86, Bu. An. Ind., 1906.

Schroeder and Cotton. Bul. 88, Bu. An. Ind., 1906.

detect the presence of tuberculosis, the method is too unreliable to be of practical value as a means of testing milk. Nowhere, as yet, has the examination of milk for the detection of the presence of this organism been undertaken as a method of municipal routine, and whether this will ever be done is doubtful. Such study, however, demonstrates beyond peradventure that the milk of tuberculosis animals may contain tuberculosis germs.

It is then clearly important to know at what stage of the disease the milk may become thus affected, and upon this subject there has been a large amount of experimenting with more or less contradictory results. If the disease is located in the udder, the milk is practically sure to be infected with tuberculosis bacilli in greater or less numbers. Perhaps 8 per cent. of the tuberculous cattle have the disease in the udder (Pearson), although some place the number lower. If the disease occurs elsewhere in the body, but not in the udder, the results are less certain. It has been shown, however, that some animals that react to the tuberculin test without showing signs of the disease in the udder produce milk containing tuberculosis bacilli. Such reacting animals clearly have the disease, but it is apparently located elsewhere in the body than in the udder; their milk may produce tuberculosis when inoculated into guinea-pigs, indicating that under some conditions animals that have no *visible* signs of udder disease may produce infected milk.¹ It is not of so much importance to settle the matter absolutely as it seems at first, because many animals are doubtless infected with the disease in the udder without showing it by any visible signs. Moreover, an animal affected with the disease may at any moment have the disease attack the udder,

¹ Rabinowitsch. *Zeit. f. Hyg.*, xxxvii., p. 439, 1901.

Mohler. *Bul. 44, Bu. An. Ind.*, 1903.

Obermüller. *Hyg. Rund.*, p. 712, 1897.

Pettersson. *Cent. f. Bact.*, xxxii., p. 274, 1903.

Beck. *Hyg. Rund.*, p. 490, 1901.

after which for considerable time she may produce infected milk without there being any visible indication that the udder has been affected. The only logical conclusion to be drawn is that any animal suffering from the disease is a source of danger, inasmuch as we cannot tell when she may begin to produce infected milk. At the same time, we must recognize that only a small proportion of the animals actually suffering from tuberculosis produce infected milk, although we have no means of knowing how large the proportion is. It is a matter of more practical importance to learn whether market milk is actually infected. A microscopic study of market milk and market butter has demonstrated beyond peradventure that the milk sold to the public is more or less frequently infected with the tuberculosis bacillus from animals. Here, again, percentages are quite impossible, because of the difficulty of the tests and the lack of a sufficient number of experiments.

One very important phase of the relation of this disease to milk distribution should be emphasized and clearly understood. The tuberculosis bacillus *does not multiply in milk*. This germ will not grow at all, unless the temperature is kept close to body heat, and it does not develop outside of the human body by any known natural media. The germs which pass from the cow into the milk will remain alive therein for a considerable length of time and are, perhaps, capable of doing injury; but they do not increase in numbers. Hence, it follows that the general mixing of milk before it reaches market is quite sure to distribute the germs somewhat widely in the different portions of the milk, and this dilution greatly decreases the number in any particular sample of milk.¹ Inasmuch as the germs do not grow, the more the infected milk is diluted with uninfected milk the more the germs will be reduced in numbers in each sample.

A second factor of no less importance is that if these germs

¹ Gebhardt. *Virch. Arch.*, p. 119, 1890.

are capable of producing tuberculosis in man—a question to be noticed presently—they will do so only when present in considerable numbers. The human body has certain powers of resistance against them, and the danger in swallowing a few is comparatively small, whereas, if large numbers are taken into the body the danger is greater. Hence, milk that contains only a very small number of these germs, scattered somewhat indefinitely, is practically harmless, and would probably never produce tuberculosis; whereas milk that contains them in larger numbers, so that a person who takes a drink of it would swallow considerable numbers of the germs, is more likely to be a source of danger.

The Use of Milk in Tuberculous Cattle.—The possibility of the distribution of tuberculosis by means of milk has a number of very practical bearings upon dairying. Experiment has shown that such milk is liable to produce tuberculosis when fed to susceptible animals. The animals that most commonly acquire the disease from milk are calves and pigs. The milk of tuberculous cows is, in many cases, the source of tuberculosis among calves feeding upon the milk. Whether calves from tuberculous mothers can be reared without danger of the disease is one that not unfrequently arises as a practical question. It has been demonstrated that, excepting in the very rarest of instances, this disease is not hereditary, and that calves when born, even though their mother is diseased, are free from it. Under these circumstances, if they can be kept later from infection, they may be raised with as good chances for health as any other calves. In order to do this, however, they must not drink their mother's milk, but must be removed from tuberculous mothers and fed only upon the milk of healthy animals, or upon milk that has been pasteurized. If this is done, there is no need of sacrificing calves of mothers that are suffering from tuberculosis.

There is little doubt, however, that by means of the milk of such animals this disease has become common in our

herds and is becoming more and more wide-spread. Creameries, skimming stations and cheese factories constitute potent agents in such distribution. These central stations receive milk from a wide territory and, having passed the milk through the separator, return to the farmers the skim milk from the creameries, or the whey from cheese factories. The routine methods in running such stations never allow a farmer to receive back his own skim milk, but he receives the equivalent amount that chances to be ready for distribution at the time he is ready to take it. The result is that the milk of any tuberculous cattle in the district will in the course of time be distributed through the whole territory. This will be followed by the presence of tuberculosis among new herds, especially of calves, and also by the development of this disease among swine that are fed upon such products. These central stations are without doubt one of the chief agents in distributing tuberculosis over the country. There is one remedy for this, and one only. The milk from such creameries should be pasteurized before it is returned to the farmer. In Denmark this is required by law, and the farmers never take back to their farm milk that has not been subjected to a heat supposedly sufficient to destroy tuberculosis bacilli. This procedure has rarely been adopted in this country, but until it is adopted no dairyman can be safe from the danger of tuberculosis among his calves and swine, so long as they are fed upon skim milk returned from the creamery or cheese factory.

Relation to Mankind.—A phase of this subject of even more general interest is the relation of the milk from tuberculous cattle to man, and the very important question whether tuberculosis among mankind is not attributable to the use of milk from tuberculous cattle. Over this question there has been in the last 10 years much dispute, many differences of opinion and a vast deal of experimenting. Out of the growing mass of facts that has been accumulating during this period have

gradually been emerging a few salient conclusions, which are now practically demonstrated:

1. The bacterium which produces this disease in cattle and in man is essentially the same. After much experimenting it has been agreed that there are slight differences between bovine and human tuberculosis bacillus.¹ These differences are in minor characteristics, which can be recognized by the bacteriologists. One of the primary differences is that in experimenting with animals the bovine bacillus proves to be, as a rule, of a more violent type than the human bacillus.² In spite of the slight differences, however, it has been proved that the two types of organisms are so closely related that the germs from either man or cattle may produce the disease in the other. Many instances have been shown where the human bacillus is capable of producing tuberculosis among cattle, although, as a rule, of rather a mild type. The experiments of a reverse nature, by which the bovine bacillus is inoculated into man, are more difficult. A sufficient number of observations along the line of accidental inoculation have shown that in some cases tuberculosis has been produced in man by the bacillus that comes from cattle.³ The general conclusion, therefore, to-day, is that while there are differences in germs from the two sources, either may be a source of danger to mankind.

2. A second question over which a difference of opinion has arisen is whether *pulmonary* tuberculosis in man is likely to be due to the germs in milk, which are taken into the stomach. The stomach would not naturally be looked on as an entrance to the lungs. But it has been shown that the mode of entrance for pulmonary tuberculosis is not necessarily, as has been frequently assumed, through the respiratory organs, but that at least in many cases, germs entering the body through the

¹ Behring, Römer and Ruppel. *Beit. z. Exper. Thierapis*, Marburg, 1902.

De Schweinitz. 17th Ann. Rep. Bu. An. Ind., 1900.

² Müller-Erfurt. *Zeit. f. Fleisch u. Milch Hyg.*, xii., p. 12, 1902.

³ De Schweinitz and Schroeder. *Bul. 52, Part II.*, Bu. An. Ind., 1905.

intestines find their way to the lungs and produce infection there.¹ Hence, the fact that milk is swallowed and not taken into the lungs is no argument that it may not be a source of pulmonary consumption in mankind. Indeed, in most recent times it has been claimed that most cases of pulmonary tuberculosis come from infection through the intestines rather than through the lungs.²

3. From these facts it has been finally agreed that the milk of tuberculous cattle is undoubtedly a source of danger to those drinking such milk. When the question of the extent of the danger is raised, however, there is still a wide difference of opinion. In the light of the facts in our possession to-day we cannot doubt that the danger exists, but whether it is a slight or serious danger we do not positively know.³ On the one hand, we find it claimed that a large part of the human tuberculosis, including pulmonary as well as intestinal, is attributable to the bacteria of the milk supply. Behring thinks that the larger part of pulmonary consumption is contracted in childhood from drinking milk, but that it may remain dormant for many years showing itself later in life.⁴ If this is the case, milk is the great source from which this scourge is contracted. On the other hand, it is held by Koch and others that this source of danger is a comparatively slight one. The great source of tuberculosis is, they hold, in breathing the germs directly into the lungs. Hence, it is the sputum of human patients rather than the milk of tuberculous cows that is the source of greatest danger.

¹ Calmette and Guerin. *Ann. d. l'Inst. Past.*, XX., p. 354, 1906.

Ipsen. *Berl. Klin. Wochensch.*, p. 791, 1906.

² Arloing. *Cent. f. Bact.*, I., xxxi., p. 255, 1902.

Zwick. *Zeit. f. Fleisch. u. Milch. Hyg.*, xvii., p. 69, 1906.

Tonzig. *Arch. f. Hyg.*, xli., p. 46, 1901.

Schroeder and Cotton. *Bul. 95, Bu. An. In.*, 1906.

³ Speck. *Zeit. f. Hyg.*, xlvi., p. 27, 1905.

⁴ Sobotta. *Zeit. f. Fleisch u. Milch Hyg.*, xvi., p. 88, 1904.

Behring. *Berl. Klin. Wochensch.*, pp. 233, 337, 689, 1903.

Behring. *Tuberkuloseentstehung, Tuberkulosebekämpfung und Säuglings-nahrung*, Berlin, 1904.

This school recognizes that for young children, who live largely upon milk and whose resisting powers are slight, milk is a serious danger, but it does not look upon it as of much danger to adults. Between these two somewhat radically different views it is too early to decide definitely. It will be recognized that the adoption of one or the other attitude will make a great difference in the methods of fighting this disease. If Behring's idea is correct, tuberculosis among mankind is to be fought by getting rid of tuberculous cows, and to do this he has devised a method of preventive inoculation, which he thinks is efficient in protecting cattle from the disease. If the other view is correct, the disease must be fought essentially as it is to-day, by protecting mankind from infection from other human patients, and chiefly from the sputum.

One or two facts bearing upon this question it is necessary to mention. Tuberculosis is common among nations drinking cow's milk, but it is also common among some nations that do not use cow's milk as a food. Japan, Greenland and Turkey, for example, do not use cow's milk but suffer from this disease.¹ It is also important to note that although tuberculosis among cattle has been on the increase in the last 25 years, tuberculosis among men has been upon an equally constant decline. This decline in the disease among men has probably been rightly attributed to improved hygienic conditions. It is certainly not due to decreased chance of contagion from tuberculous milk. It is interesting to note that the decrease in the disease has not affected the intestinal tuberculosis among children, a form which, more than any other, would naturally be attributed to milk.

In the light of all these facts it must be admitted that the milk of tuberculous cows is a source of danger, although we may not yet agree as to the extent of the danger. However great or however small the danger may be the desirability of

¹ Heyman. *Zeit. f. Hyg.*, xlviii., p. 45, 1905.

guarding against it is evident enough. The milk of our markets certainly contains tubercle bacilli. In Berlin 30 per cent. of the market milk contains them, and it is not likely that our markets are in much better condition. Every dairyman should recognize the force of the question how he may best protect not only his own herds from this menace, but the health of his patrons from the menace of the disease through the milk of his cattle. The first safeguard to be taken is to use every means to free his herd from the disease. The methods of doing this are given in other places and cannot be dwelt upon here.¹ The only way that a dairyman can be sure that no dangerous tuberculous milk is furnished to his patrons is by having all his herds properly tested with tuberculin, and so treated as to be sure that none of them are suffering from the disease.

The fact that such large numbers of animals suffer from tuberculosis has made it hitherto practically impossible to bring about an elimination of all tuberculous animals from the milk-producing herd of the public. The expense of destroying all tuberculous animals has been quite prohibitive. Under these circumstances milk derived from tuberculous animals has been and still is being used beyond doubt. Such milk can be rendered perfectly safe, however, by the process of pasteurization, which will be referred to later. It should, perhaps, be stated here that no bacteriological nor chemical analysis and no system of dairy inspection yet devised can thoroughly guard the public milk supply so far as concerns the distribution of tuberculosis. Until a law requires the universal testing of all cattle and the immediate slaughter of all reacting animals any public dairy inspection will be futile in this respect.

¹ Harding, Smith and Moore. Bul. 277, N. Y. Exper. Sta., Geneva, 1906.

OTHER DISEASES DERIVED FROM THE COW

There are no other diseases to which milch cows are subject that we know positively are liable to infect the milk to any considerable importance. Animals with *foot or mouth disease* or *splenic fever* will give infected milk, and man might take the disease, but these diseases are sudden and violent, and are, moreover, at least in this country, quite rare. *Lockjaw* and *hydrophobia* might sometimes be transmitted through milk. But the violence of the diseases and the extremity of the sickness will hardly ever make it possible that the milk of such animals should reach the public for consumption. At all events we have at the present time no suspicion that the public is in any source of danger from the milk from such animals, and this subject may be passed with the simple caution that no animal suffering from any violent disease should be allowed to furnish milk for public consumption.

DISEASES DERIVED FROM EXTERNAL SOURCES

After the milk is drawn from the cow there are, at least, three kinds of disease germs that may and sometimes do find their way into it and become a source of danger to the consumer.

Typhoid Fever.—The first and most important of these is the *typhoid fever* germ.¹ (Figs. 38 and 39.) Typhoid fever epidemics attributable to milk are fairly common and, within a comparatively few years, at least fifty have been thus traced. The general character of such an epidemic is sharp and violent but short. There will usually appear within a few days numerous cases of fever; but after a week or two further new cases become less common and soon disappear. Such a brief

¹ Freeman. Med. Record of N. Y. Mar., 1896.

Fulton. Jour. of Hyg., i., p. 422, 1901.

Rembold. Cent. f. Bact. I., xxxiii., p. 204, 1903.

epidemic gives every indication of being confined to a single instance of infection. They differ notably from typhoid fever epidemics due to the water supply in the fact that the latter are much more lasting, continuing for weeks and months. No milk epidemic has, as yet, been known to last long, and all seem due to one brief period of infection. The explanation of this is natural. The chance of infection of milk with typhoid fever bacteria is always a remote one, and it is never likely to continue for any considerable length of time. In other words,



FIG. 38—TYPHOID BACILLI
Ordinary culture
Flagella not shown
(Lehmann & Neumann)

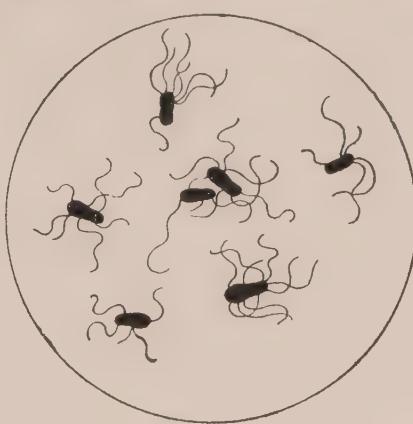


FIG. 39—TYPHOID BACILLI
Showing flagella
(Lehmann & Neumann)

the infection is confined to one or two days only, after which the milk from this same source usually becomes normal again. A single day's infection will produce the short, sharp type of epidemic above referred to.

We must next ask for the source by which milk becomes infected with typhoid fever germs. It must first be recognized that only one source of typhoid fever germs is known, viz: the intestinal contents of a typhoid fever patient. They pass out of the body in the faeces or from the mouth and are distributed by various means. The methods by which they get into the milk are, of course, not always the same, and they may be grouped under three heads:

1. Direct contact with a patient suffering from this disease. Sometimes patients carry these germs for months and even years after recovery from the disease. Such "chronic bacilli carriers" are a constant menace when employed in the dairy.¹ Typhoid fever, as is well known, does not always confine a patient to his bed, and sometimes it is of a mild enough character to allow him to continue for days, or even weeks, about his ordinary occupations. Such cases are called "walking typhoid." He frequently may not know that he has typhoid fever; but during all this period he is discharging typhoid germs from his body and is liable to infect anything he touches. If such a person is employed in a dairy and touches the milk cans or handles the various utensils, the chance of the germs from his hands or clothing entering the milk is very great. Some of the typhoid fever epidemics have been traced to such causes.

2. The patients who are sick enough to be confined in bed may, in an indirect way, be the cause of an infection of the milk. Discharges from such a patient always contain typhoid fever bacteria in abundance. These discharges may be carelessly handled by persons who are employed in the dairy, or such persons may soil their own hands with the soiled clothing or bedding from typhoid patients. Subsequently they may milk the cows or handle milk cans or other utensils. Here is evidently an easy source of infection. The only safeguard against this chance of trouble is the absolute prohibition of anyone who has anything to do in the dairy having any connection with the sick room of a person suffering from intestinal fevers. Some health inspectors now forbid the sale of milk from any farm or dairy where there is a case of typhoid or other infectious disease.

3. Infected water is in some cases a source of trouble. The discharges of typhoid fever patients thrown upon the ground, or otherwise improperly disposed of, may percolate through

¹ Lentz. Hyg. Rund., xvii., p. 377, 1907.

the soil with the drainage, or may run over the surface with rain-water and eventually find their way into wells, brooks or rivers. Sewage frequently drains directly into rivers. The water thus becomes infected with the typhoid bacilli, which may remain alive for some time; subsequently such water may infect the milk in several ways. If the milkman has the dishonest practice of watering his milk, the chance of contagion is evident. But it is also a common practice for the dairyman to cool milk by submerging the milk cans in cold water nearly sufficient to cover them. Under these circumstances, the cans become covered with water containing typhoid fever germs, and there will occasionally be an opportunity for some of them to pass into the milk pails, so that the milk will thus be infected.¹ It is even possible for such contaminated water to get into the milk cans in the course of washing the cans. If milk cans are washed in water that has not been boiled, or are rinsed in cool water, or if they are washed with cloths that have been rinsed in cold water, there is always a possibility of the introduction of typhoid germs into milk. One very severe typhoid epidemic at Stamford was caused by rinsing cans in cold water after washing them in hot water.² Lastly, cows wading in such polluted water may get the typhoid germs on their udders and teats, from which entrance to the milk is easy. One typhoid epidemic³ of 43 cases was traced to the use of whey from a cheese factory.

It may seem that the chance of infection from these sources is remote, and that under no conditions could many bacteria find their way into the milk. These are undoubtedly correct inferences, but in dealing with typhoid fever we have one source of danger that does not occur in the distribution of tuberculosis. *Typhoid bacteria multiply in the milk.* As a result, if from any one of these sources a very small number, perhaps even

¹ Sedgwick and Chapin. *Bost. Med. and Sur. Jour.*, p. 129, 1893.

² Smith. *Rep. of the Stamford Typhoid Epidemic*, New Haven, 1895.

³ Dabney. *Phila. Med. News*, 63, 1893.

half-a-dozen, of these microscopic germs make their way into the milk, they find the milk such a favorable condition for growth that they multiply rapidly and, in the course of a few hours, become indefinitely numerous. By the time this milk has reached the consumer they may have become so abundant as to constitute a very considerable source of danger. It is beyond doubt this multiplication of bacteria in the milk that explains the violence of the milk typhoid fever epidemics. Wherever the germs are present at all they are likely to be abundant from this growth, and under these circumstances a large proportion of those drinking the milk are sure to be infected. In this respect the conditions are very different from the distribution of the typhoid by water. In water the multiplication of the germs is slight, and they rarely become very abundant. As a rule, water epidemics of typhoid, though wide-spread, affect only a small portion of those drinking the water, while a much larger proportion of those drinking milk are thus infected. It is, therefore, of the utmost importance for the dairyman to remember that typhoid must be absolutely excluded, for the entrance of a very few germs is a source of danger; a condition quite different from that of tuberculosis, where the presence of a small number of germs in the milk is not a source of danger to anyone. The milk has much less chance of becoming infected with typhoid bacteria than with tuberculosis germs, but when it does occur the danger to the consumer is very much greater.

Since typhoid fever epidemics due to milk are frequent, health officials are sometimes called upon to trace their origin. The steps in such a problem are simple. In tracing the source of a typhoid epidemic the first step is to determine the limits of the disease, and if possible to find whether the cases occur in such a way as to exclude the water supply as a source of contamination. If all of the cases occur rapidly, new cases failing to appear after two or three weeks, a single temporary

source of infection must be looked for. This will commonly exclude the water supply, since if a water supply is infected the trouble is apt to last a long time and will never be confined to one single period of infection. The next step will be to determine whether all persons suffering from the disease have had any one point of contact, as having attended a banquet, for example, at any particular time. If so, the disease may be traced to something used during the banquet. If no such common point is found, the next point of suspicion would be the milk supply, and the question would be raised whether all of the persons suffering from the disease had obtained milk from a common source. If this is found to be the case, the matter is practically demonstrated, and it is only necessary to trace the milk to its origin, and find the methods by which it is distributed. There is usually little difficulty in detecting the source of contamination, either from a typhoid fever patient in the dairyman's family or employ, or from an infected well or brook, from which water has come into contact with the milk cans.

Diphtheria.—A few epidemics of this disease have been attributed beyond doubt to milk.¹ In these cases the infection occurs after the milk has been drawn from the cow, although



FIG. 40—DIPHTHERIA BACILLI
(LEHMANN & NEUMANN)

¹ Abbott. Jour. Path. and Bact., II., p. 35, 1894.

Dean and Todd. Jour. of Hyg., II., 1902.

Klein. Cent. f. Bact., vii., p. 489, 1890.

Mackenzie. Med. News, p. 180, 1893.

Marshall. Jour. of Hyg., vii., p. 32, 1907.

some have believed that the cow herself may suffer from the disease and affect the milk through the udder. No such instances are positively known, but it is known that secondary infection is possible. It is well known that convalescents from this disease carry active germs in their throats for some time after they appear perfectly recovered. (Fig. 40.) Such persons would naturally be allowed to return to work in the dairy, and the chances of infection in such instances are very great. Although such instances are not common they have been known; and the milk supply must be looked upon as one of the sources of the distribution of this disease.¹

Scarlet Fever.—Scarlet fever has almost exactly the same relation to milk as diphtheria. We have as yet no proof that the disease may come from the cow, although one epidemic of a somewhat similar disease has been attributed to diseased cows in Herndon, England, and other cases have been reported of a similar nature. There is fairly good evidence that the milk may become infected by persons who have the disease or are recovering from it. When such persons have direct or indirect contact with the dairy there is occasional opportunity for the milk to become infected. The cause of this disease is as yet unknown, but it is doubtless some living germ. At all events those who subsequently consume such infected milk do sometimes contract the disease. A few epidemics have been traced sufficiently to convince us that here is a positive source of danger, though perhaps not a very great one.

Asiatic Cholera is also transmitted by milk, but this is a matter of no special significance in the United States.

¹ Davies. *Jour. of Hyg.*, p. 338, 1901.

Klein. *Cent. f. Bact.*, ii., p. 219, 1887.

Lee. *Hyg. Rund.*, p. 227, 1900.

Tingvall. *Hyg. Rund.*, p. 109, 1904.

DETECTION OF DISEASE GERMS IN MILK

There are within our reach at present no practical means by which we can examine milk and determine whether it contains the germs of any of these four diseases. It is true that proper study will sometimes detect the presence of tubercle bacilli in milk; but the methods are long, complicated and very difficult, and so uncertain that a negative result has no meaning. It would never be possible by any methods in our possession to-day to make a microscopic examination of milk and guarantee the milk as being free from tubercle bacilli. It is still more impossible to detect the presence of the other three disease germs. The cause of scarlet fever is not known and cannot, of course, be recognized in milk. The typhoid bacillus very closely resembles *B. coli*, which we have seen is always to be expected in milk, and while the two bacteria can be distinguished from each other, the methods are too long and uncertain to make it possible to apply them in any practical way to the study of milk. The diphtheria bacillus, too, may be detected but only by methods of no practical value in the study of milk. Moreover, no sample of milk is likely to be thrown under suspicion as distributing disease until long after the particular sample is consumed, when it is too late to make any study.

It is important to notice that the numbers of bacteria in milk have no relation whatsoever to its chance of distributing these four diseases. Milk that contains as many bacteria as 100,000,000 per c.c. is no more likely to contain these germs than milk that contains not more than 10,000, excepting on the general ground that the milk with the higher numbers has been perhaps more carelessly produced. In short, the plans of municipal inspection which give us facts concerning the bacteria in milk give us not even a hint as to the germs that cause these four diseases. Furthermore, dairy inspection by any means as yet devised is not accurate enough to enable the inspector to de-

termine whether tuberculous cattle are present among the herds furnishing milk to the public, or to guarantee the consumer against contagion from typhoid or diphtheria from all the sources above mentioned. The sources of infection from typhoid fever, diphtheria, scarlet fever or tuberculosis are varied and so rare that dairy inspection will give little protection against them. A rigid dairy inspection, it is true, forces the dairyman to be more careful in the handling of his milk and more cleanly in his general methods; but this will not guarantee that the tuberculosis bacillus is not constantly finding its way into the milk, nor will it insure that typhoid fever germs are never present. On general principles the most carefully kept dairy would be least likely to furnish milk infected with disease germs. So far, then, as dairy inspection can bring about this desired result, it will reduce the danger of contagion; but since even well kept dairies have in some cases been known to distribute milk contaminated with disease germs, there is no method at hand by which it is possible for the public to be guaranteed that the milk which comes from the dairies is free from the germs of these four specific diseases. In other words, as valuable as dairy inspection may be, it must be recognized that it is inadequate at the present time as a guard against tuberculosis, typhoid fever, scarlet fever and diphtheria.¹

DIARRHEAL DISEASES

This type of disease has quite a different relation to our problems. *Diarrheal diseases* are somewhat indefinite, the term including such common troubles as *summer complaint*, *cholera infantum* and other miscellaneous types of intestinal troubles common in warm weather. The causes of these diseases are, as yet, unknown. They are not specific diseases like the four

¹ Bienstock. *Arch. f. Hyg.*, xxxix., p. 390, 1901.

Eichert. *Zeit. f. Fleisch u. Milch Hyg.*, viii., p. 86, 1898.

Lubbert. *Zeit. f. Fleisch u. Milch Hyg.*, vii., p. 1, 1896.

Neumann. *Zeit. f. Fleisch u. Milch Hyg.*, vi., p. 53, 1894.

already mentioned, and none of them can probably be attributed to any particular type of bacteria. Delepine has concluded that they are sometimes due to bacteria of the type of *B. coli*, found in the faecal matter¹. The organism that he thinks is the cause differs from the ordinary *B. coli* in producing a small amount of acid and in not curdling the milk, and is frequently distributed by milk. Lameris has concluded that diarrhea may be produced by a Streptococcus that comes from the udders of cows suffering from *mastitis*, and inflamed udders have certainly been known to produce milk that gives rise to diarrhea.² It has been shown that such milk may still produce the trouble, even after boiling, and this and other general facts have led to the belief that this type of disease is of a toxic rather than a bacterial nature.³ By this is meant that the direct cause is some substance of a poisonous nature that is swallowed with food or drink, and directly poisons the individual. This does not mean that bacteria are not the real agents, but that they act in an indirect way. In the case of tuberculosis the germs enter the body and live within the living tissues as parasites, but in the toxic diseases the bacteria grow in the food or drink, and there produce poisons. When these foods are subsequently swallowed they, with their poisons, will be absorbed from the digestive tract and cause a direct poisoning. If this is true, it will follow that toxic poisoning would result from the use of food only after bacteria have had an opportunity to develop sufficiently to produce their toxins. It would follow, therefore, that their frequency would be proportionate with the number of certain species of bacteria in the foods.

With these facts in mind the relation of such troubles to milk becomes suggestive. While there are other sources for

¹ Delepine. *Jour. of Hyg.*, p. 68, 1903.

² Lameris. *Zeit. f. Fleisch u. Milch Hyg.*, xi., p. 114, 1900.

³ Gröning. *Inaug. dis.*, Bern, 1901.

them it appears that they may in part be attributed to milk bacteria.¹ They seem to be associated with large numbers of bacteria in milk. They are especially common among children in warm weather, and the mortality from such diseases rises and falls with the number of bacteria in milk. This in itself does not prove much, however, for the number of bacteria in milk simply rises and falls with the temperature, and it may be that temperature itself is the primary factor. But it becomes more significant when we learn that these diarrheal troubles during the severe warm weather are far less abundant among children that are breast-fed, thus obtaining their milk from a source where the number of bacteria will be comparatively small, than among those fed on cow's milk. Again experience has shown that pasteurizing the milk, which reduces the number without destroying them all, causes a notable reduction in the amount of intestinal disturbances and in the mortality of children from such sources. Lastly, among children fed upon certain improved forms of milk, which we shall notice later, the death rate from such causes is notably less than among children fed upon ordinary milk. In our large cities the children's death rate is startling in hot weather, and is always directly associated with a poor quality and a high bacterial content of the milk.

TOXIC POISONING FROM MILK PRODUCTS

Closely related to the last topic are the occasional instances of toxic poisoning from milk, cream or cheese. These are characterized by violent cases of illness that follow the consumption of a single lot of poisonous food and come to a crisis and pass off rapidly. A few instances of such severe illness due to drinking a lot of milk² have been recorded and several of similar

¹ Rickards. *Jour. of Hyg.*, 1903.

² Zammet. *Brit. Med. Jour.*, p. 2054, 1900.

Vaughan. *Arch. f. Hyg.*, xxvii., p. 308, 1896.

results from eating poisonous cheese.¹ Cases of poisoning from eating ice cream are still more common. In all these instances certain kinds of bacteria have developed in the food until they have produced a considerable quantity of toxin, sufficient to bring about violent illness to the person swallowing it. The poison that causes the trouble has been separated from cheese and called *tyrotoxicon* by Vaughan, and he has also found in some samples of ice cream the species of bacterium that produces the toxin.

The only way of protecting milk from this trouble is to use it fresh, for it will never appear until the bacteria become very abundant. No means can be given for avoiding poisoning in cheese except that of using only good milk. We know so little as to the matter that we are as yet helpless in regard to it. Probably the use of lactic acid starters in cheese-making, which is becoming common, will do much toward eliminating such troubles which, fortunately, are very rare.

Ice Cream Poisoning.—Instances of this nature are more common and are probably more easily avoided. They occur almost always in hot summer weather and are apparently most liable to occur in the hot days that follow a period of cool weather. That they are attributable to certain poisons in the ice cream produced by the growth of bacteria is beyond question. It has been shown above that if the milk is kept cool for a number of days the lactic acid bacteria fail to grow; but at these low temperatures other kinds of bacteria do grow, even though somewhat slowly. If the cream collected from day to day is kept for a number of days in the cool temperature of an ice chest, there will be a continuous growth of these other types of germs which are not checked by the development of the lactic bacteria. During a period of cool days there is no demand for ice cream; the cream accumulates in the ice chest,

¹ Vaughan. Med. and Sur. Reporter, Ixiii., p. 584, 1890.

Vaughan. Med. News, II., p. 644, 1887.

and these bacteria that love the cool temperatures are growing all the time, so that in the end they will be present in large numbers and will produce a quantity of toxic products. Now, if the cream is made into ice cream when the next hot days appear, the toxins which have been developing may cause among those eating the cream a violent intestinal disturbance. Little more is known concerning this trouble than the facts here stated. The cause is probably the development of toxic germs in cream kept at fairly low temperatures unchecked by the growth of lactic germs, which would at a higher temperature have prevented their development.

It must be emphasized, however, that if these troubles do come from such a source, it is not the mere presence of *numbers* that produces trouble. As already mentioned, sour milk and buttermilk are wholesome products and have never been suspected of causing diarrheal troubles; yet they contain bacteria in prodigious quantities. From this it will follow, of course, that there are some kinds of bacteria whose presence in milk is deleterious and others whose presence is harmless. What types of bacteria are concerned in producing these toxins, we do not know at present. It is a growing belief that the bacteria concerned belong to the putrefying class. Putrefying bodies certainly do frequently show the presence of toxins, and it would be natural to assume that milk in which such germs are growing would contain enough of the poisonous bodies to produce a toxic poisoning in children. We have already learned that the putrefactive bacteria frequently liquefy gelatin and are consequently easily detected on common gelatin plates. Hence, we should be led to throw under suspicion any milk that shows large numbers of liquefiers. On the other hand, it is quite certain that the lactic acid bacteria are not responsible for these troubles. Indeed, the presence of lactic acid bacteria prevents the growth of the putrefactive forms, and when the acid type becomes very abundant, as high as hundreds of millions per c.c.,

the milk may for this very reason be perfectly wholesome, since the putrefactive bacteria have not been able to grow sufficiently to produce any considerable amount of toxin. On the other hand, another sample containing smaller numbers may be liable to produce intestinal disturbances, because the acid bacteria have not developed sufficiently to prevent the growth of the toxic forming species. Sour milk is practically sure to contain few putrefactive bacteria, if the lactic acid type is abundant.

Evidently the toxic poisoning and the diarrheal troubles are closely related. In the former a large amount of poison is developed and swallowed at one time, producing violent results; in the latter the toxins are in smaller quantity but may be taken time after time in successive meals, producing a long continued effect.

CHAPTER VI

DAIRY METHODS

WE are now prepared to understand the meaning of various dairy methods which have been devised for improving the conditions of milk. To appreciate these it must be remembered that milk is an ideal medium for bacterial growth. It is a good food, is well supplied with water and is, when drawn from the animal, at a temperature best calculated for the most rapid growth of nearly all types of bacteria. It should also be borne in mind, that the bacterial contamination of milk is mostly external to the cow, and that although some of the bacteria of milk come directly from the udder and are unavoidable, the vast majority are from external sources, which can be more or less controlled. All reforms in the dairy are aimed, therefore, to accomplish three ends: 1. To insure the use of none but healthy cattle. 2. To protect the milk as far as possible from external contamination from bacteria after it leaves the cow's udder. 3. To produce conditions which so far as possible prevent the growth of these bacteria. In the outline which we will give of methods to be adopted for these purposes, we shall first explain all of the improvements which have been proposed in dairy methods for improving the milk product; but since it is impossible for most dairies to adopt them all, we shall in the next chapter summarize briefly the more important of the practical plans that may be adopted for producing a general improvement in milk without involving very great expense.¹

¹ Fraser. Ill. Exper. Sta., 1903.

Fraser. Bul. No. 92, Ill. Exper. Sta., 1903.

Marshall. Bul. No. 28, Mich. Exper. Sta., 1905.

Haecker and Melick. Bul. 87, Neb. Exper. Sta., 1905.

Doane. Bu. 88, Md. Agri. Exper. Sta., 1903.

The dairyman may feel that some of the precautions are unnecessary, and that so many changes from old methods are needless. But he should remember that conditions are rapidly changing and that there is a much more emphatic demand for cleanliness in milk than ever before. He should also remember that it is exactly these precautions that will enable him to guard the butter and cheese-maker from troubles which we shall notice later. The demand for greater care in the production of milk is a growing one, and one that will be more and more rigidly enforced.

AT THE BARN

The Health of the Cow.—This hardly belongs to our immediate subject, but it is so closely related to the wholesomeness of milk that it cannot be passed unnoticed. A veterinarian should periodically inspect the cattle, to determine their soundness; and no animal suffering from any udder disease should be allowed to contribute to the milk supply. The importance of excluding tuberculous cattle from those contributing to the public milk supply cannot be over-rated. The only method by which this can be done is not only to have an inspection of all the cattle, but to have at intervals a tuberculin test applied to the whole herd to detect this disease in any animal. Any animal that reacts must be separated at once from the rest of the herd, and her milk must not be sold. A universal tuberculin test of the cattle in the country is not possible under present conditions, but it must be emphatically insisted that so long as the cows that furnish milk to the public are not thus inspected and tested, the public is in a constant source of danger from tuberculosis. Careful watch should be kept of the udder of the animals, and whenever any signs of udder disease appear in the form of inflammation or hardness, running sores or the appearance of bloody milk, the animal should be excluded at

once from the milk-producing herd until complete recovery has taken place. It is well also to remember that exercise is needed by the cow, both for its own health as well as for that of human beings, and that the best of results can never be obtained unless the cows are given a certain amount of outdoor exercise.

The Stables.—The *location* of the cow stables should be such as to give the freest possible circulation of air. (Fig. 41.) It is much better to have them on high ground in which there is ready drainage than on low ground. Both *air* and *light* in the



FIG. 41—MODEL DAIRY BARN

stables are necessary for the best results. Each cow should have three to four square feet of window surface and 400 to 450 cubic feet of air space. If possible a separate place for calving should be provided. While the animals are in the yard, as they should be daily, the stables should be thoroughly aired. (Figs. 42 and 43.) The older plan of keeping the barns tightly closed for the purpose of saving warmth injures both the quality of the milk and the health of the cattle. A good system of ventilation is necessary.

The *cleanliness* of the stable is a matter of utmost importance. The habits of the cow and the nature of the manure are such

as render a high state of cleanliness very difficult. But the dairyman should understand that all accumulation of manure or other filth is a direct detriment to the quality of the milk. The cleaner the stable the better the result. (Fig. 44.) The removal of the manure from the stalls should be as frequent as possible. In the better kept dairies this is done several times a day, although upon the ordinary farm it is less frequently attended to; but it should never be less than twice a day. The more frequently it is removed, and consequently the cleaner the



FIG. 42—POORLY-KEPT BARNYARD, WET AND FILLED WITH MANURE

cows and stalls are kept, the better the results on the milk product. The manure, when removed, should be taken as far as possible from the barn and should not be heaped outside of the barn close by, nor be allowed to accumulate in the cellar. By far the best method is to distribute it daily upon the fields, where it is to be used as a fertilizer. If this daily spreading is impracticable, it is advisable to remove it to some distance from the barn as often as opportunity permits, always remembering that the greater the accumulation of manure the worse the results on the milk. Attention should be given to the *dust, cob-*

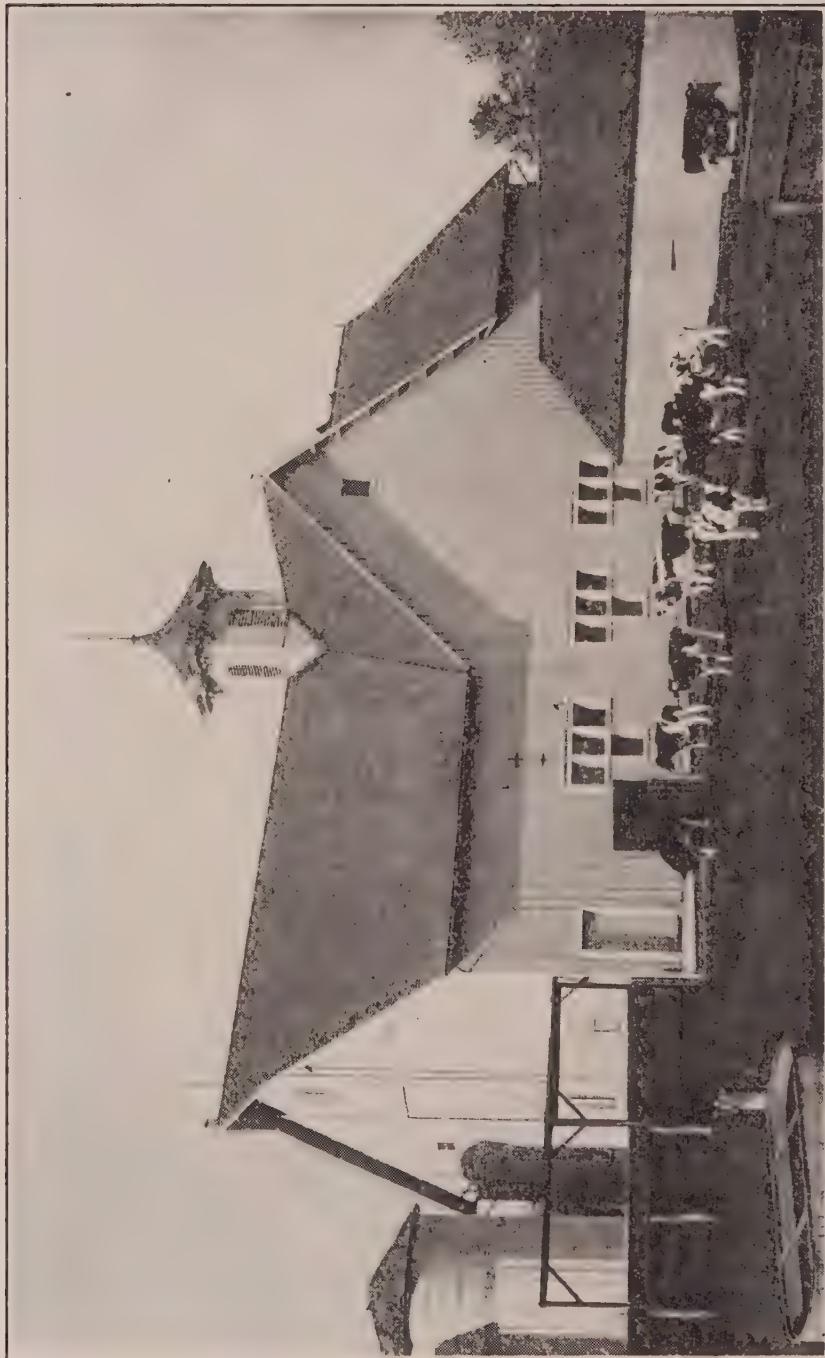


FIG. 43—WELL-KEPT, DRY BARN YARD



FIG. 44—INTERIOR OF A WELL-EQUIPPED, WELL-KEPT COW BARN

webs and *hay* that may be clinging to the ceilings of the barn, for all such are traps for accumulating dirt, as well as sources of bacteria, thus aiding in contaminating the milk. Plastered or sheathed walls and ceilings are very much to be preferred to a rough finish. The bedding of the cattle is a matter of some importance also, and clean shavings appear on the whole the best for this purpose. They serve better than straw or hay,



FIG. 45—COLONIES

Developing from bacteria falling upon a plate exposed during milking in a poorly-kept barn (Fraser)



FIG. 46—COLONIES

Developing from bacteria falling upon a plate exposed during milking in a well-kept barn (Fraser)

inasmuch as they give rise to less dust and dirt and are not so liable to be a source of bacteria contamination for the milk. A coat of *whitewash* should be applied with a spray pump at least once a year. It helps to disinfect and makes the stable light and clean. Flushing with water should be frequent. (Figs. 45 and 46.)

DAIRY PERSONNEL

There has been a strong tendency in recent years to give much greater attention to the habits of the persons employed on a dairy farm. It is not uncommon to find them dressed in

white clothes, which are better than colored clothes because they show dirt when soiled. The milking clothes should be made of washable materials. These are worn during the milking or handling the milk, and some dairies insist that this clothing should be sterilized each day. Directions for thorough washing and drying the hands before milking, and for the care of the person are coming to be common in the better class of dairies.



FIG. 47—MILKING IN A MILKING ROOM
An ideal condition of cleanliness

We have already learned that bacteria from the persons employed in the dairy are more likely to be dangerous than those from any other source. For all of these reasons it is desirable that proper care should be given to persons employed in the dairy. No one should be allowed to handle any milk, to wash the milk cans, or to handle the milking utensils in any way, who is suffering from any contagious disease. Nor indeed should any farm furnish milk to the public if there is a case of typhoid,

scarlet fever or diphtheria among its employees, unless a health inspector pronounces the sanitary conditions satisfactory.

THE MILKING ROOM

A separate milking room is a rarity in our dairies, but from the standpoint of the purity of the milk such a room, separate from the stalls where the cows are kept, is eminently desirable.



FIG. 48—MILKING IN A MODEL DAIRY BARN

In the **places** where this has been tried, it has produced an improved quality of the milk. The reason is evident enough. Since the cows are only taken to the room for the few moments of the milking, it is possible to keep this room in a condition of far greater cleanliness than the cows' stalls can possibly be kept. (Figs. 47 and 48.) The cows soon adapt themselves to the new routine, yield their milk readily in the milking room,

and dairymen who have used separate rooms for this purpose have been highly satisfied with their utility. There are, of course, objections. More space is required if the cows are to be milked in a separate room, and it is more trouble to remove the cows from their stalls and take them to the milking room and subsequently lead them back again. The plan thus involves some expense; but the extra work certainly results in an extra purity of milk, and is one of the devices that should



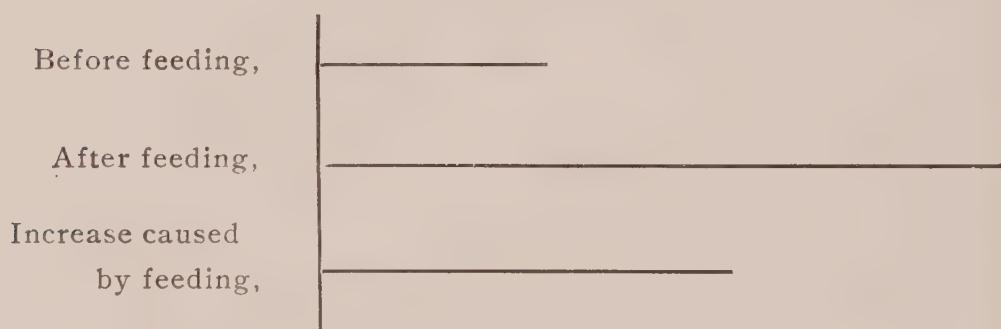
FIG. 49—AN IDEAL METHOD OF MILKING

be adopted where one is attempting to obtain the highest grade of product. Where possible, milking out of doors is to be especially recommended. (Fig. 49.)

During the milking process the cows should not be allowed to feed, nor should the feeding precede, but rather follow the milking. The feeding of the cows is almost always sure to fill the air with more or less dirt and dust from hay and dry grain. Experiment has shown that a great number of the bacteria in milk may come from the feed of the cows, if the feeding has occurred before or during the milking. If fed immediately after, the bacteria have a chance to settle to the floor before the next milking and do not produce so much trouble. The

effect of feeding cows upon dry corn stover before and after milking is shown by the following diagram:

DIAGRAM SHOWING THE EFFECT UPON THE BACTERIA OF FEEDING CORN STOVER



The milking room should, if possible, be flushed with water after each milking. There should also be flowing water, soap and towels to permit the milker to wash his hands after milking each cow, and whenever they are soiled.¹

THE COW

Emphasis must be placed upon the *grooming* of the cows. In earlier years it was almost unknown to pay any attention to the grooming and cleaning of the cattle. (Fig. 31.) Fortunately, there has been a decided change in this respect, and at the present time cattle in dairies are not infrequently groomed and brushed, and are sometimes kept in a condition as cleanly as that of the ordinary horse. If a proper milk supply is to be obtained, this is absolutely necessary. The habits of cows, and especially the close confinement in which they are kept in stalls, inevitably results in a large amount of manure adhering to the animal's flanks, tail and udder, and unless this is removed by curry comb and brush, with washing if necessary, the character of the milk is sure to suffer. The long hairs of the cow's body are especially liable to catch such filth, and the cutting off with

¹ Stocking. Bul. 42, Storrs Exper. Sta., 1906.

scissors or a clipper of the long hairs from flanks where manure is liable to collect, from the tip of the tail, and especially around the udder and on the under part of the body, is to be recommended. If this hair is occasionally cut, the contamination of milk from the cow's body is lessened. The cow should not be allowed to wade through mud and manure such as is frequently found in the barnyard. This will mean the cleaning of the barnyard and, in many places, a thorough draining. A clean, dry barnyard for the cattle to use for exercise will contribute much to the cleanness of the cows and hence of the milk. (Fig. 43.)

MILK VESSELS

Perhaps the most important factor for producing a reduction of bacterial contamination is the proper cleaning of the milk vessels. From facts given in a previous chapter it is evident that all vessels which have held milk should receive a most thorough cleaning. This refers to milk pails, strainers, coolers, separators, milk cans, glass bottles and, in fact, to all dairy utensils. The washing of such utensils is no easy task, and it is not an exaggeration to say that upon an ordinary milk farm vessels are never washed clean. They are, it is true, frequently scrubbed with warm water and soap, but no such washing will result in removing all of the bacteria. After the most thorough washing and scrubbing there will still be left many of these organisms in the cracks and clinging to the milk vessels, ready to begin feeding upon the next lot of milk and to multiply. Cold water is practically useless for removing them. All milk vessels should be of metal, with a smooth surface. If the coating of tin is worn off, they should be discarded, for they cannot be kept clean. They should not be allowed to become dry before washing, for dried milk is difficult to remove. They should be first soaked in warm water to loosen the milk; then thoroughly washed in hot water, preferably containing soap or sal-soda,

and thoroughly scrubbed; after this a second rinsing in hot water is extremely desirable in order that the milk pail shall be cleaned in fair condition. After washing they should be placed inverted in the sun until used again. They should not be tightly closed. Such a washing as this is about all that they can receive upon an ordinary farm, but this is not enough to sterilize them.

There is, indeed, no means by which the farmer can so thoroughly wash his milk cans as to get rid of all bacteria. Every creamery, cheese factory or separating station should understand this and appreciate that a satisfactory washing can be accomplished only where there is a supply of steam. Hence, *no creamery should depend upon the farmer to wash milk cans.* Where a supply of steam is to be had a sterilization must follow the washing, but it should be different from that frequently seen. The common procedure is to place the can for a few seconds over a jet of exhaust steam, and after a short time to remove it that it may be replaced by a second. The steam used under these conditions hardly heats the can hot enough to kill any bacteria, and while cans thus steamed are certainly somewhat more free from them than those not thus treated, the impression of the dairyman that he has thoroughly cleaned his cans of bacteria is incorrect. The only method by which milk vessels can be absolutely freed from bacteria is by thorough sterilization. This can be done by steam if the cans be placed in a steam chamber built for the purpose, and the steam turned on and left to act upon the cans for an hour or so. In no other way can absolute sterilization be accomplished. Washing with hot water is better than with cold, washing with sal-soda is better than without it, subjecting them to a jet of exhaust steam is better than simple washing, and sterilizing in a steam chest is better than all. Each dairyman should adopt as thorough cleaning as is practicable.

In washing dairy utensils special attention should be given

to *separators*, which are a very important source of bacteria. These are frequently insufficiently washed, particularly hand separators. They are difficult to clean adequately, since it is commonly not easy to steam them. For this reason all the more care should be exercised in using plenty of boiling water and soap, and steaming all parts that can be steamed.

Caution should be given that all pieces of *cloth* used around a milk room need frequent sterilization. A neglect of this precaution has sometimes caused serious troubles in making starters in butter or cheese-making, and keeping undesirable bacteria away from them.

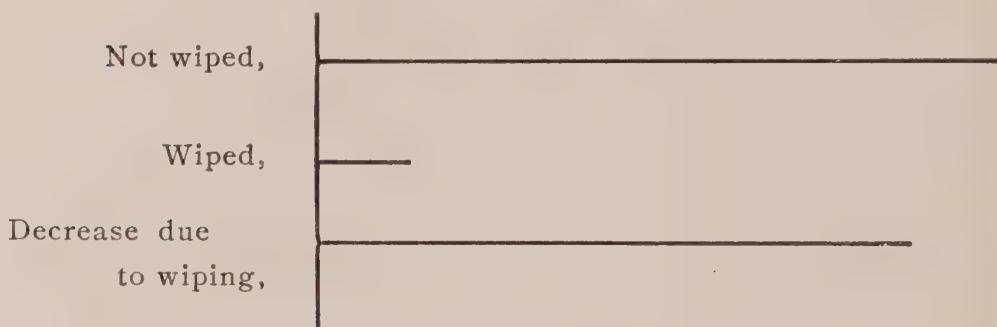


FIG. 50—METHOD OF MOISTENING COW'S UDDER DURING MILKING (FRASER)

THE MILKING

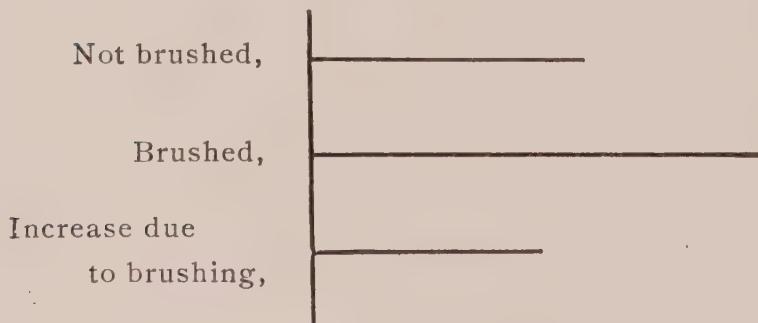
Moistening the Udder.—The value of moistening the udder with a damp cloth or sponge before milking is greater than one would imagine. Its purpose is, of course, to prevent the fall of dirt into the pail. It may be done with vaseline or a borax solution, but plain water serves just about as well. The udder should not be left wet but simply damp. No simple procedure is more efficient than this in reducing bacterial contamination. (Fig. 50.) The value of such treatment in reducing the number of bacteria is shown in the following diagram:

DIAGRAM SHOWING THE EFFECT UPON THE BACTERIA OF MILK OF
WIPING THE UDDER WITH A DAMP CLOTH



Some dairies have the practice of *brushing* the cow just before milking. This by no means serves the purpose of wiping with a damp cloth. On the contrary it simply stirs up the dirt and dust and leaves it floating in the air in a position to settle readily into the pail. The result is that there is an actual increase of bacteria rather than a decrease. The amount of increase, as shown by one series of tests, is indicated in the accompanying diagram.

DIAGRAM SHOWING THE EFFECT OF DRY BRUSHING OF THE UDDER
BEFORE WIPING



The Covered Pail.—In very recent years changes have been made in the methods of milking, designed for reducing the amount of dirt which falls into the milk during the milking process. The first and simplest of these is the change in the

style of milk pail. The old fashioned pail had a flaring top, the purpose of which was to make the milking as easy as possible, but incidentally it resulted in exposing the milk to much contamination by dirt and bacteria. Various devices for protecting the milk from such exposure by the use of covered milk pails, are now used. Figures 51 and 52 show some of these devices. There is quite a great variety among them, but they all have the general plan of decreasing the size of the opening of the milk vessels so as to expose less surface for the entrance of



FIG. 51—COVERED MILK PAIL (NORTHS)

dirt, and they also have in the opening some kind of a cloth *strainer* for catching the larger particles of dirt and keeping them from the milk. The pail of Gurler uses a layer of absorbent cotton for the purpose. Such milk pails produce a decided improvement in the milk. Careful testing has shown that by their use a large proportion of the dirt that gets into milk may be avoided. Their use has been extending rapidly, and the covered milk pails will soon doubtless take the place of the old flaring tops. Every dairyman should recognize this as *one of the easiest, cheapest and most efficient means* at his disposal for improving the character of his milk. It is necessary to use

them intelligently, however, for a careless milker can obtain as bad results from a covered pail as from any other. In their use special attention must be given to the cloths used for strainers. These must be sterilized daily by washing and boiling. It is sometimes customary to change the strainer cloth for a fresh one between the milking of each two cows. Strainers



FIG. 52—COVERED MILK PAILS (GURLERS AND STADTMULLERS)

made of bolting cloth, Irish linen, or flannel are the best, and these may be used over and over again with the proper washing. If absorbent cotton is used for a strainer, it must be thrown away after the milking.

Milking Machines.—A still more recent means of reducing contamination is by machines designed to convey the milk directly from the cow's teat to closed sterilized milk cans, without allowing it to come into contact with the air, or exposing it to contamination with dirt.¹ These milking machines consist of rubber tubes ending in special cups for attachment to the teats of the cow and connected at the other end with large cans, which can be sterilized. (Fig. 53.) The cans are connected with a vacuum system of tubes, and at the point where the rubber tubes are attached to the can there is a mechanical device

¹ Lane and Stocking. Bul. 92, Bu. An. Ind., 1907.

by which the vacuum is made to draw through the tubes intermittently, thus imitating natural milking. (Fig. 54.) This draws the milk through the tubes to the can. Details of these machines need not concern us here, but their efficiency in milk-

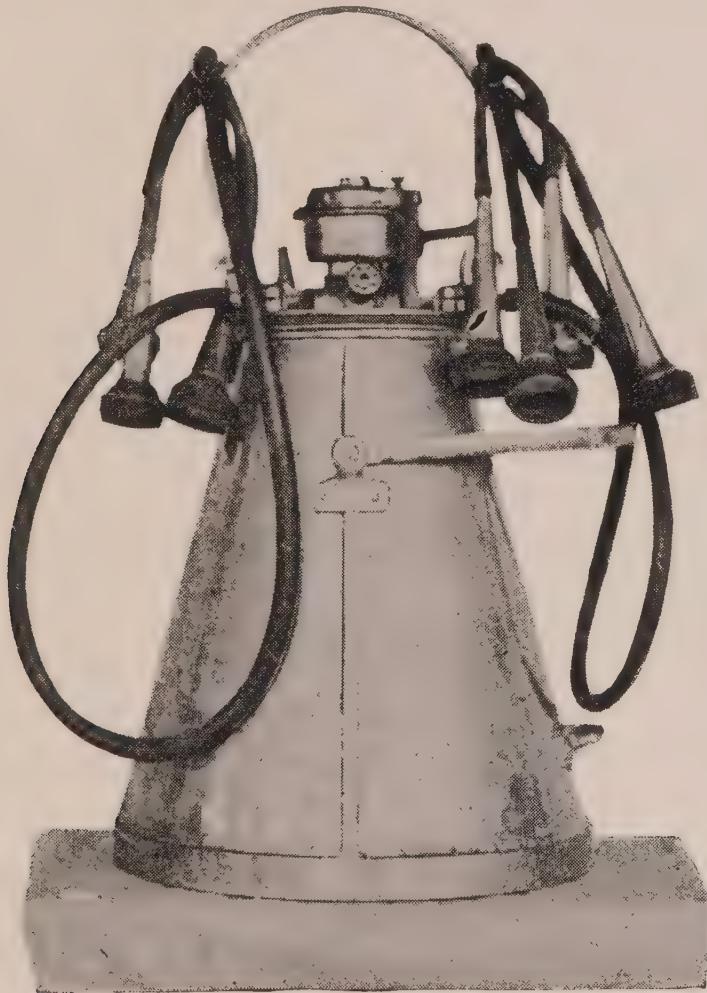


FIG. 53—MILKING MACHINE
(United States Department of Agriculture)

ing is beyond question. It would seem that such a plan, which carries milk directly from the teat to a sterilized can, without contact with the air, would be almost ideal and would remove practically all dirt contamination. All ordinary sources of filth contamination are excluded by such milking machines, and

where they have been intelligently used they have been found to be efficient in producing a much cleaner quality of milk. On the other hand, they are themselves difficult to clean. Long rubber tubes which have been filled with milk are by no means easy to keep sweet, and when they are used by careless employees the bacteria become very abundant inside the tubes and the



FIG. 54—USE OF A MILKING MACHINE OUT OF DOORS
Power is supplied by animals in treadmill on right
(Kansas Experiment Station)

other parts of the somewhat complicated machine. In other words, it requires very great care in cleaning and sterilizing these milking machines in order to produce better results than are produced by hand milking, or even as good; but they can be cleaned, and, if care is taken to sterilize thoroughly all of the apparatus, better and more reliable milk can be obtained by the use of the milking machine. Directions for cleaning these machines will always be given with the machines themselves and need not be given here. The general principle, however, that extreme care should be taken to disinfect thoroughly the rubber tubing after each milking cannot be too highly emphasized. The use of these milking machines certainly offers a great

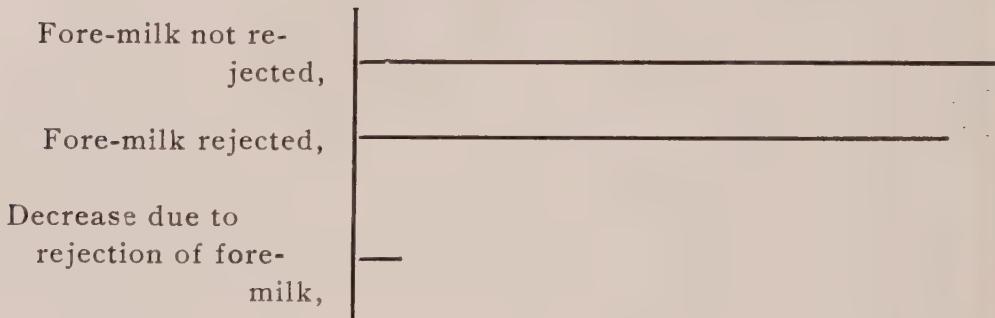
promise in the milk product. They are hardly practical, however, at the present time upon the small farm, because of their expense. But in a dairy where there is a herd of fifty or more cows they become very economical because of the ease with which a single man can milk a number of cows at once by means of them.

One serious objection has been raised against the use of these machines that has in some places caused their abandonment. There is no way yet devised by which the milker can see the milk as it passes into the collecting can. If he chances to milk a cow that has experienced some accident so as to produce bloody milk the milker will not discover it until he opens the can into which he has perhaps already drawn the milk of several cows. The result will be the spoiling of the whole milk in the can which would have been avoided if he had been able to detect the bloody milk at the time of the milking.

Rejecting of Fore-milk.—For reasons already indicated the first milk drawn at each milking will contain more bacteria than the rest, inasmuch as it washes out the bacteria at the mouth of the milk ducts. It has become a practice in the better dairies to reject the fore-milk, either allowing it to waste upon the floor or collecting it in a separate dish. There is no doubt an advantage in this, but the extent of the advantage has been overdrawn. The extra number of bacteria obtained in a pail of milk from the entrance of the fore-milk is very small in proportion to the larger number that enter the milk from other sources, and while the rejection of the fore milk is to be recommended its value is not so great as is sometimes supposed. Its value is shown by the accompanying diagram.

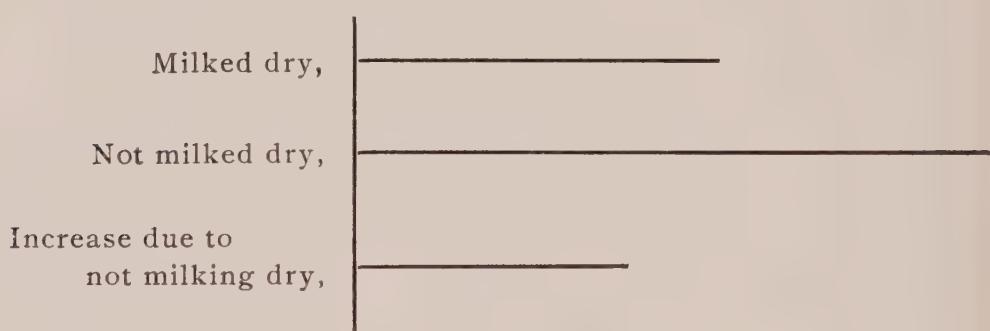
Stripping.—The practice of milking the cows thoroughly dry at each milking also tends to increase the purity of the next milk drawn. If a considerable quantity of milk is left in the udder, it has a tendency to hasten the development of the bacteria in the udder so that the next milk that is drawn con-

DIAGRAM SHOWING THE EFFECT UPON THE BACTERIA REJECTING THE FORE-MILK



tains more than it otherwise would. The practice should, therefore, be adopted of milking the cows as dry as possible. Its value is shown by the accompanying diagram.

DIAGRAM SHOWING THE EFFECT UPON THE BACTERIA OF STRIPPING

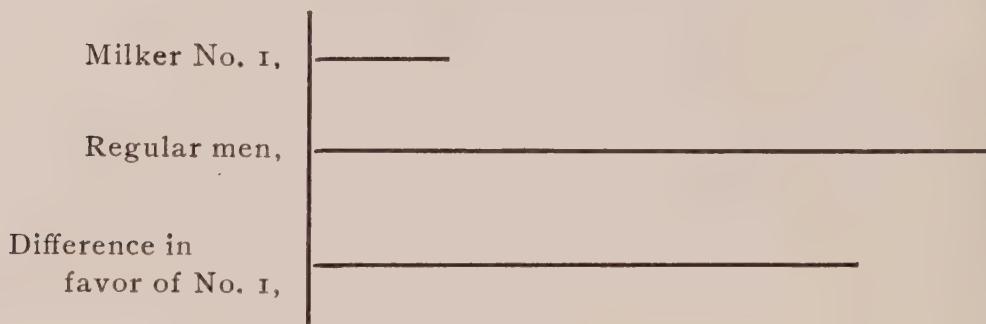


Removal from the Milking Room.—All milk should be removed from the milking room immediately after milking, and the quicker the better. The air of the room is sure to be filled with bacteria or dust, which will get into the milk. Moreover, the odor in the milking room is commonly quite noticeable, and it is well known that milk absorbs odors rapidly. Accordingly if the milk stands in the barn, it will collect an appreciable odor, and one that decidedly detracts from its value. Hence, the more quickly the milk is removed the better; and it is best to have a collecting can outside of the milking room and to empty the

milking pail between the milking of every two cows into the receptacle outside. The value and necessity of this procedure will, of course, vary with the cleanliness of the milking stall itself.

The Value of the Trained Dairyman.—Apparatus without a proper man to use it is valueless. It makes no difference how many rules may be drawn concerning the dairy, how complicated the apparatus becomes, or how careful the directions given to the employees; it is quite impossible to expect satisfactory results without properly educated and trained assistants. An untrained man will only succeed in getting bad results, even with the best of apparatus. Special emphasis, therefore, should be placed upon the matter of the necessity of *education*. It must be recognized that the employees in our dairies at the present time are, in many cases, without any proper training. They do not know the character of the product they are producing; they do not know the dangers to which it is subject; they do not understand the universal presence of bacteria; they do not understand, in general, the problems that are concerned with their business. They are quite likely to believe the whole subject of bacteria in milk to be foolishness and not worth their attention. Under these circumstances, no matter how many directions are given, or how much instruction there may be, satisfactory results will never be obtained. We may look with more confidence upon the dairy conditions after the next few years. At the present time a large number of young people are being trained in the necessary knowledge to enable them successfully to handle the problems of dairy work. Only as more education is spread will it be possible to bring about the desired end in the dairy industry. The great significance of this matter of intelligent training is shown by the accompanying diagram. In a barn were employed a number of regular milkers, who had no special knowledge of bacteria, and one person, who had studied bacteria so that he understood the principles concerned.

DIAGRAM SHOWING THE VALUE OF TRAINING THE MILKER UPON THE NUMBER OF BACTERIA



Both were supplied with the same apparatus for milking, but the student used it more intelligently. The length of the lines shows the relative number of bacteria in the milk drawn by the regular milkers and the trained student. The result is striking and most emphatically shows the emphasis that is to be placed on the proper training for men employed in the dairy.

AT THE DAIRY

Location.—Thought and care should be given to the location and arrangement of the dairy. It should be in a light, airy location, unconnected with stables or living rooms. The floor should be of cement with good drainage. Windows should be abundant to let in plenty of light, covering a space equal at least to 15 per cent. of the floor space. There should be a satisfactory plumbing system both for furnishing an abundant supply of clean water and for carrying away wastes, the latter being as important as the former. That a dairy should be kept clean hardly needs emphasis, but it cannot be too strongly brought to the attention of all interested in the handling of milk. Constant cleaning of all utensils and vats, floors and walls, with hot water and soap, and steaming of all cans, bottles or pails in which milk is placed must be recognized as a necessary condition for a properly kept dairy that shall furnish the best of milk. No

milk spilled on the floor should be allowed to sour, but should be washed up at once. No dairy is properly equipped unless it has an *ice supply*. The amount of ice needed will vary with the climate, being, of course, less in colder than in warmer regions. For an ordinary climate at least 40 lbs. of ice are needed for every hundred pounds of milk produced.

Cooling.—The importance of cooling the milk, and cooling it *immediately*, cannot be over-stated. As we have already seen, bacteria grow very readily at a temperature of the animal's body, very slowly at temperatures below 50 degrees, and scarcely at all at the temperature of freezing. When the milk is drawn from the animal it is, therefore, in a condition to stimulate the growth of bacteria to its utmost. It is true that for a while, because of the germicidal property of milk, the bacteria do not grow; but this condition lasts only a short time, after which, if the milk is warm, they begin to develop with the greatest rapidity. If the milk, however, has been in the meantime cooled to a low temperature, the bacteria that have found their way into the milk will not grow rapidly, and the milk can be kept for a very much longer period. These facts are so simple as hardly to require statement, but, unfortunately, many a dairy-man, although he may theoretically understand them, fails to appreciate their importance. It not infrequently happens that a milkman finds that his morning's milk sours when delivered to his customers more quickly than milk of the night before, and he has been puzzled over the matter. The reason usually is that the night's milk, after milking, is placed at once in a cool place, an ice chest or a cold stream, and by morning it has acquired a fairly cool temperature. In the haste of the morning, however, the milkman does not cool his morning's milk but carries it to market in a warm condition. By the time it reaches the customer the bacteria have begun to develop. In the meantime the milk of the night before has not been warmed up sufficiently for the bacteria to begin to grow rapidly. Thus, the

morning's milk soon contains many more bacteria than the milk that is twelve hours older, and hence it sours much more quickly. The lesson to be drawn from this is the necessity of *immediately cooling* the milk to a temperature as low as 40°, if possible. It should be remembered, too, that it is just as necessary to cool good milk as it is to cool dirty milk. Unless this is done the good milk will soon contain as many bacteria as the dirtiest milk.

It should be recognized, however, that the value of the cooling of the milk is only to decrease the rapidity of the multiplication of the bacteria and does not necessarily affect its healthfulness. The milk, as we have seen, may distribute the germs of certain diseases. If these germs have found their way into the milk, the fact that the milk has been cooled will not destroy them and will have no value in rendering the milk wholesome. None of the disease germs known to be found in milk would be killed, or even injured, by the temperature of the ice chest, at least if continued only for a few hours, and therefore the practice of cooling the milk cannot be looked upon as a safeguard for protecting the public from the specific diseases liable to be distributed by it. On the other hand, the development of the diarrheal diseases which, as we have learned, probably come from poisons secreted in the milk by certain kinds of bacteria may, perhaps, be checked by the use of cool temperatures that will prevent the growth of bacteria. Concerning this we know practically nothing at the present time. The value of the immediate cooling of the milk, therefore, is primarily in making the milk keep longer, and not in rendering it more wholesome. It decreases numbers only and does not remove disease germs.

Any method that cools the milk rapidly is satisfactory. It may be done by immersing it, already bottled, into cold water. This has the advantage of cooling it rapidly in the jar so that no further disturbance of the milk is necessary. Cooling in cans is less desirable since the milk must afterward be handled again.

A common method of cooling in large dairies is by causing it to run over a metallic surface that is artificially cooled. This cools and aerates the milk at the same time.

Aeration.—The practice of aerating at the time of cooling the milk has become very general in the better dairies, and it is undoubtedly of considerable value. It is accomplished by allowing the milk to flow in a broad stream and a thin layer over artificially cooled metallic surfaces. So far as concerns its effect upon the bacteria of milk it is of no value at all, for it does not decrease the numbers but, on the other hand, tends slightly to increase them. It is easy to see that if the milk is for some time exposed to a large surface of air, it will be constantly absorbing bacteria. The number thus obtained, however, is not great, and more probably come directly from contact with the aerator itself. Both together do not furnish enough to overcome the advantage to the milk from aeration. It is, however, claimed by Marshall¹ that aeration increases the subsequent rapidity of bacteria growth, since it removes the carbon dioxide that is normally present in the milk which has a slight checking action on bacteria growth. This has, however, been questioned by others. The purpose of the aeration is both to cool the milk by contact with cold surfaces and to remove some of the so-called "animal odors" that are commonly present. It should be noticed that most of the "animal odors" are purely filth odors, due mostly to manure. Milk that is drawn under perfectly clean conditions, without chance for contamination or entrance of manure, does not need aeration for the purpose of removing odors. Such milk is best bottled *at once* without aeration and cooled in the bottles. The need of aeration is directly proportional to the amount of filth that enters the milk after it is drawn. If aeration is adopted it should be carried on in a place where the air is as pure as possible. To aerate in a room where

¹ Marshall. Ann. Rep. Mich. Bd. of Agri., p. 259, 1902.
Barthel. Rev. Gen. d'Lait, III., p. 294, 1904.

the dust of the barn has a chance to enter is bad. Aeration improves the flavor of milk that is produced under any but the very best of conditions, but it does not decrease the number of bacteria and is of no value to milk that is to be used in butter making or cheese making, except for its rapid *cooling* of the milk.

Filtering Milk.—The practice of filtering milk to remove the dirt has been adopted in some places, especially in Europe. The filter is commonly made of layers of sand of different degrees of fineness, and in the best methods the milk is forced through the filter from below upwards. These filters are not very widely in use. So far as bacteria are concerned they do not accomplish anything, for they are never fine enough to remove them. They do remove some of the dirt. They are difficult to clean thoroughly and are not, in general, to be recommended.¹

Centrifugal Force as a Method of Cleaning Milk.—In some dairies the use of centrifugal machines has been adopted for cleaning milk. In doing this the rapidity of the rotation of the machine is not as great as when it is desired to separate the milk from the cream. Careful testing shows that this treatment certainly does remove a large part of the dirt from the milk. One who examines the mass of sediment that collects in the drum of such machines cannot doubt that a large amount of dirt is removed. Further, an examination of the milk itself and an estimation of the milk which it holds shows that there is a noticeable reduction. Curiously enough, however, such a process does not reduce the number of bacteria. It certainly would be expected that, if it removes dirt, it would at the same time reduce the bacteria. On the contrary, the number of bacteria usually appears to be increased by passing through the centrifugal machine, and is practically never reduced. The reasons

¹ Weigmann. Milchztg., p. 289, 1901.

Weil. Milchztg., p. 739, 1901.

Vieth and Martiny. Zeit. f. Fleisch u. Milch Hyg., XI., p. 326, 1901.

for this are probably three. 1. The centrifugal machine itself is somewhat complicated and is extremely difficult to get thoroughly clean. After using, it is, of course, washed, but it is difficult or impossible to wash the bacteria all away; and when the next lot of milk is passed through it, there may be some contamination of milk by bacteria from the machine. Probably, however, this is a small item. 2. In the method of determining bacteria, as shown on page 273, the number is determined from the numbers that grow in gelatin, and it is assumed that there was in the original milk one bacterium for each colony. It not infrequently happens that bacteria are just about to divide, and if such a half-divided bacterium is in the milk that has been tested, the two halves being so closely together will make only one colony, and will be counted as one, whereas they will be broken apart by the centrifugalization and appear as two colonies. 3. It frequently happens that several bacteria may cling together in the original milk, when the whole bunch would produce a single colony and be counted as one. When, however, milk is passed through the centrifugal machine the agitation it receives is apt to break the bacteria apart and thus increase the number of isolated individuals. The result would be a larger number of colonies in the gelatin plates, and these when counted would give a greater number of bacteria in the milk passed through the machine. Hence, although centrifugalization cannot really increase the number of bacteria, nor does it improve the keeping quality of the milk, it does increase the number counted on a gelatin plate. In spite of this fact, it is beyond doubt a very useful means of cleaning milk, since milk that has been thus centrifugalized is deprived of most of its filth.¹

¹ Dunbar and Kisler. *Milchztg.*, p. 753, 1899.

Rolet. *Rev. Gen. d'Lait*, I., p. 185, 1901.

Eckles and Barnes. *Bul. 59, Iowa Exper. Sta.*, 1901.

Severin. *Cent. f. Bact.*, II., xiv., p. 605, 1905.

INFECTION AT THE CREAMERY AND FACTORY

The primary infection of milk is at the barn and dairy, but a secondary infection may occur elsewhere, either in creameries or factories or milk distributing stations, and it is necessary to guard milk in these places. For example, many creameries that handle milk cans are careless about their washing, and even if they thoroughly wash them, will only adopt the system of a steam jet for a few seconds for the purpose of sterilizing. Many factories do even worse than this; they allow dirty, unwashed cans to lie around the factory for some time or permit the farmers to carry them away unwashed. Any factory that *depends upon the farmer to wash the cans will suffer for it.* The factory cans circulate from one farm to another, and if a farm once becomes contaminated with bacteria, such trouble is sure to be distributed through the factory to the whole community. The remedy is to cut it off at the creamery, and this can be done only by thorough cleaning and sterilizing of the cans at this central station.

In many separating stations and cheese factories a very unfortunate custom has arisen that distributes all kinds of bacteria and makes it *impossible* for the dairyman to furnish a good product. The farmer brings his milk in cans and carries back to his farm whatever skim milk chances to be running through the separator at the time. By the time he has reached home, especially in summer, the skim milk is apt to be sour. The farmer may now attempt to wash the cans after emptying them, but this is really impossible. The cans cannot be properly cleaned on the farm, and fresh milk is put into them at the next milking. Under such conditions it is impossible to produce good milk. The conditions are still worse in some cheese factories, where the farmer takes back *whey* to his home. The whey from the cheese vats is stored in tanks or barrels from which the farmer fills his milk cans. The whey tanks are not washed,

and this whey is frequently in an advanced state of fermentation. It is sure to pollute the milk cans beyond the possibility of cleansing by any ordinary washing. Under such conditions trouble is sure to arise, and by this means not infrequently such infections as slimy milk, or other mischievous fermentations, are widely spread. The remedy is either to furnish separate receptacles for returning the by-products to the farm, or to pasteurize them before giving them back to the farmer. The latter is by far the better and is already adopted by law in some countries. In all cases the whey should be returned to the farm as quickly as possible. The creamery should be the *pattern for the farmer*, but, alas, it is sometimes only a pattern of carelessness and filth, and the individual dairies will always be worse than the creamery.

In the disposal of wastes, too, many factories or creameries are greatly at fault. The practice of allowing any kind of waste to accumulate close to the factory is undoubtedly a fruitful cause of trouble. The draining systems should have plenty of running water to carry away all wastes and to empty them at a considerable distance from the creamery. Dirty cans, or cans filled with refuse from the milk, should not be allowed to stand around the rooms where fresh milk is placed, or upon platforms in contiguity with cans containing fresh milk. The water which is used in factories for cooling milk should be as fresh and clean as possible, and any kind of dirt in it is greatly to be deprecated. It must always be recognized that although water is used to cool the can only, and is not meant to touch the milk, it is quite impossible to keep cans of milk in water and handle them as rapidly and as roughly as they have to be handled without allowing the water to splash upon the covers, and hence to get into the milk itself.

The milk supply company in the city is frequently at fault for troubles which it attributes to the farmer. It is not uncommon to find the cans in which milk has been shipped to the

city simply emptied of milk and shipped back again without washing. By the time they reach the farm they are in a very bad condition, smelling strongly, filled with dried milk and bad condition of filth. Such dirty cans a farmer *cannot possibly clean* and even if they are sent to a creamery where there is plenty of live steam the thorough cleaning is extremely difficult and almost impossible in the few moments that can be given to each can. The next milk that goes to the city in these cans is badly contaminated even at the start and cannot reach the city in good condition. The city distributing company blames the producer when the real trouble is the failure to wash the cans clean at the time they are emptied. All cans should be *washed in the receiving station as soon as they are emptied* and shipped back in a clean condition. That this rule is not followed any one may prove to himself by simply looking at and smelling of the empty cans he may find at a railroad station.

DISINFECTION OF THE BARN OR DAIRY

It sometimes happens that a disinfection of the barn or dairy is a matter of necessity. This may be due either to the appearance in the milk of some unusual or troublesome fermentation, like slimy milk, which the dairyman finds difficult to eradicate by ordinary means, or it may be necessary to rid the premises of the germs of some contagious disease. The thoroughness of the disinfection needed will be dependent upon the circumstances of the case, and it should be done with especial care where it is necessary to get rid of some contagious disease.

Disinfection of dairy premises is not an easy matter, and to do it thoroughly is extremely difficult. If a *barn* is to be disinfected, one must first remove all filth by scraping, and thoroughly clean the floors, walls and ceilings by the use of hot water, scrubbing completely from the floors and cracks all filth that may be accumulated. After thorough washing some dis-

infectant must be applied. In choosing the disinfectant for the purpose one must be guided by conditions. One of the best is *corrosive sublimate*, $\frac{1}{4}$ oz. to two gallons of water; but this is intensely poisonous, and is unsafe to use where cattle are to be subsequently stalled, unless absolutely thorough washing with water follows the use of the sublimate. Corrosive sublimate also corrodes metals badly and cannot be used upon metallic surfaces. *Chloride of lime*, 6 oz. to a gallon of water, is an extremely efficient disinfectant, its disinfecting power being due to the chlorine which it contains. It is less harmful than *corrosive sublimate*, but its odors make it sometimes more objectionable. No other disinfectants are as satisfactory as these two. A partial disinfection, which is sometimes quite sufficient, may be obtained by applying a thorough coat of whitewash, which may be put upon the walls, ceilings and floors with a spray pump. This also makes the room lighter and brighter, and is eminently useful. Indeed, all barns should have a coat of whitewash applied, at least twice a year, if they are to be kept in proper condition.

Disinfection of the *dairy* proper follows the same general methods described above, excepting that the premises are kept cleaner, so that the original cleaning and scraping are commonly not so necessary. The use of the corrosive sublimate is especially undesirable here, and the use of chloride of lime must be followed by thorough airing to remove the odor before the dairy is used again. If the nature of the room is such that it can be tightly closed, it may be disinfected by formalin. The application of the formalin is rarely advisable, excepting in the hands of someone who thoroughly understands it, and the details of the method need not be given here. The treatment of dairy utensils by boiling water and steam is usually sufficient, and if thorough, quite sufficient for practical disinfection.

CHAPTER VII

TREATMENT OF MILK FOR MARKET

IT IS impossible to make any statement as to the number of bacteria liable to be found in milk. There may be as few as 3,000 or as many as 200,000,000 per c.c. Even in fresh milk the number varies widely, as we have seen, and curiously enough wide variations may occur on the same farm, under apparently similar conditions. Sometimes a dairy that is producing milk regularly with few bacteria will, in one or two days, find the number enormously increased, without being able to find any explanation for the fact. The number found at any later hour in any sample is still more variable and will depend upon three factors: 1. The cleanliness of the conditions under which the milk was produced. 2. The age of the milk. 3. The temperature at which it has been kept. Of these three factors the latter is most important in determining the numbers.

There has grown up in recent years a strong demand that the number of bacteria in milk offered in market shall be kept low. While the presence of large numbers is liable to a very erroneous interpretation, no doubt it is desirable to keep the bacteria down to as small a number as possible. The question how this can be done is forcing itself more and more on the attention of the milk producer. We have noticed in the last chapter the various devices which are being adopted in dairies for reducing the original bacterial contamination. We have next to notice methods adopted for placing milk containing as few bacteria as possible in the hands of the consumer.

TRANSPORTATION

The problems of transportation resolve themselves into two: 1. Temperature. 2. Protection from contamination. The various devices by which these ends are reached cannot be considered at length here. In this country milk handlers have learned to use ice freely, and by means of it milk may be transported for long distances upon the railroad and still be fairly cool and sweet when it arrives at its destination. The ice car has made this practicable. To get the best results the milk should be cooled to as low a temperature as possible before being placed in the car, and then kept cold during transportation by the use of plenty of ice.

It is hardly necessary to say that the cooler the temperature maintained the more satisfactory the result. In transportation the milk is commonly sent in cans. For practical reasons of economy this has seemed to be necessary for large supplies of milk, although it would be a far better plan to transport it in glass jars, if this were feasible. The cans used in transportation should have a tightly fitting cover, which overhangs the top of the can and thus prevents the entrance of dirt. But the cans ordinarily used are themselves the source of much trouble and the origin of much of the bacterial contamination of the milk. A glance at the battered, rusty and dirty milk cans that one finds at a railroad station will convince him that not a little of the bacterial content of city milk comes from transportation. Of course, the expectation is that these cans will be washed before milk is placed in them again, but battered, rusty cans are practically incapable of being washed clean by any method adopted in an ordinary dairy. Cleaner cans, more frequent replacing of old with new ones, would do much toward improving the condition in which the milk reaches the city.

In the handling of the milk after it reaches the city there is another chance for contamination. The story is simply that

of the dairy repeated, and the same precautions are necessary. Cleanliness and sterilization must be the watchwords. The use of glass jars is a very great improvement over the older



FIG. 55—RESULTS OF THE ROUGH USAGE ON THE RAILROAD
It is almost impossible to clean the can on the left

method of delivering from cans, chiefly because they are sure to be cleaner. They are smooth so that they are easily washed and transparent so that dirt is quite sure to show. Then, too, after they are once filled and covered with their caps they are quite well protected from external contamination on their journey from the factory to the consumer. All of this contributes to the purity of the milk. Of course, all glass bottles must be thoroughly washed and sterilized.

INFECTION IN THE HOME

The consumer of the milk needs a caution concerning his method of keeping it as well as does the farmer for producing it. It makes no difference how fine a quality of milk may be furnished, it will not remain in good condition unless properly treated. In many cases faults and complaints that arise from the consumer are due to his own carelessness and not to the character of the milk which he receives. He places milk in a pitcher that has not been carefully washed and that still contains some bacteria from the last sour milk which it held. The milk is placed in a warm room and allowed to stand for some hours before being placed in an ice chest. If placed in an ice box, this is presumably filled with ice, but the ice frequently runs low, and the temperature rises. Under such conditions, especially in summer weather, trouble is sure to rise, and the milkman will receive the complaint that the milk will not keep when the trouble is due entirely to the treatment from the customer. For his own advantage the customer should be careful that the milk vessel in which the milk is placed has been thoroughly cleaned, that it is kept cold, that it is not allowed to become warm under any condition, and that it is not placed in a position where dirt or bad odors can reach the milk. If these suggestions are followed, the complaints of bad milk will certainly be lessened.

THE USE OF PRESERVATIVES

The use of preservatives in milk is at the present time to be unhesitatingly condemned. Whether in time perfectly harmless preservatives may be discovered cannot be predicted. But in this country the use of preservatives is, at present, illegal and cannot be recommended under any circumstances. It is, therefore, unwise here to give any directions as to the use of such materials.

The preservatives that have been most commonly used for the purpose may, however, be briefly mentioned. The most common in use in this country is *formalin*. This is a clear liquid, which is a very efficient germicide, and is the basis of most, if not all, of the liquid preservatives on the market under different names. It is very efficient as a preservative of milk, even as small a quantity as one part in 40,000 (one teaspoonful to 15 gallons of milk) noticeably enhancing its keeping property.¹ Whether this small amount injures the digestibility of the milk is not yet settled,² although in general formalin makes proteids difficult to digest, even one part to 2,000 being said to do so. But at all events formalin is an adulterant and, as such, forbidden by law in most places in the country. It is easily detected in milk by chemical means.³ *Borax*, *boracic acid* and *salicylic acid* have also been used, but they are all even more objectionable than formalin. Most of the preservatives of the market put up in powdered form contain one or more of these substances.

The use of *hydrogen peroxide* (H_2O_2) has been very strongly urged in recent years as a preservative in milk.⁴ This material, when added in small quantity, about one gram per liter (1 oz. to 9 gals.) has the advantage of rapidly disappearing from the milk, especially if subsequently heated.⁵ After being in the milk for a little, one atom of oxygen that it contains is loosened and passes off, leaving the substance as plain water H_2O . Meantime it has destroyed most of the bacteria. It seems to be an efficient and perfectly harmless preservative. It is,

¹ Lowenstein. *Zeit. f. Hyg.*, xlviii., p. 239, 1904.

Schaps. *Zeit. f. Hyg.*, I., p. 247, 1905.

² Chester and Brown. *Bul. 71, Del. Exper. Sta.*, 1905.

Trillat. *Rev. Gen. d'Lait*, III., p. 380, 1904.

³ Eury. *Milchw. Zent.*, iii., p. 173, 1907.

⁴ Budde. *Nord. Mejeri. Tidn.*, 1903.

Barthel. *Nord. Mejeri. Tidn.*, 1893; also *Rev. Gen. d'Lait*, II., p. 289, 1903.

⁵ Rosam-Pilsen. *Cent. f. Bact.*, II., viii., p. 769, 1902.

Lukin. *Cent. f. Bact.*, II., xv., p. 20, 1905.

however, expensive, and it has not yet been brought into practical use anywhere. It has hardly been tried in this country.

The use of oxygen for disinfection has also been strongly recommended. This gas, when passed in quantity into milk under proper conditions, serves as a partial disinfectant. Oxygen gas is, of course, perfectly harmless, and, moreover, it rapidly passes off from the milk, so that it can hardly be called an adulterant. Its use would, therefore, seem to offer little objection, if it can be found to be both practical and cheap. No practical method of using it to purify a milk supply has as yet been offered to the public, and none is in use at present, but it offers promise for the future.

STERILIZATION

A few years ago it was thought that the solution of the milk problem lay in destroying bacteria by heat; and there developed rapidly a system of milk sterilization. This was first recommended by physicians as possibly making the milk more digestible by cooking, and subsequently was strongly advocated by bacteriologists for destroying bacteria and rendering milk perfectly safe.

By *sterilization* is meant the treatment of milk to a temperature sufficient to destroy *all* bacteria. The word, however, has been used in two different ways, which has given rise to a slight confusion. In order to absolutely sterilize milk it is necessary to use a temperature higher than that of boiling water, for milk is practically sure to contain spores of bacteria that cannot be killed by simple boiling. In order to destroy them a temperature of 220° to 230° is necessary, and to obtain this the milk must be heated in sealed chambers under steam pressure. Only milk so heated can really be called *sterilized*, and this milk is rarely found outside of a bacteriologist's laboratory. In common use, however, the word *sterilizing* refers to milk that has been simply *boiled*. The boiling of the milk

destroys most of the bacteria that fail to produce spores. The acid bacteria are thus killed since they are not spore producers. Such boiled milk will keep for a much longer time than ordinary milk. Inasmuch, however, as it contains bacterial spores these will in time develop and cause its destruction. The milk is, therefore, not really sterilized. It should be called boiled milk, but in popular language the word sterilization is frequently applied to simple boiling.

At one time there was a vigorous attempt made to place upon the market *absolutely sterilized* milk. This product was sealed in bottles and subjected to heat of steam under pressure, and since it was sterilized it was supposed to keep indefinitely. Milk sterilization has undergone a variety of changes in public opinion, but at the present time it has become almost wholly abandoned as a method of treating milk on anything like a large scale. The sterilization of milk produces a number of chemical changes, which seem to interfere with its digestibility, so that the weak stomachs of infants and invalids cannot so easily digest and assimilate it. While many experiments have shown that sterilized milk is capable of perfect digestion by strong, vigorous persons, the evidence is fairly abundant that for weak stomachs it is not as satisfactory a food as raw milk.¹ Sterilized milk, moreover, has the taste of boiled milk, which is unpleasant to many people. It has also been found that in spite of the high heat and the greatest care even such highly heated milk is not always sterilized and, hence, will not keep.² Tests of the milk from many companies showed none in which the milk could be relied upon as always sterile, and if it could not be depended upon, the value of the process was practically nothing.³

¹ Doane and Price. Bul. 77, Md. Agri. Exper. Sta., 1901.

Dukes. Lancet, p. 1857, 1901.

Variot. Rev. Gen. d'Lait, iv., p. 238, 1905.

² Flügge. Zeit. f. Hyg., xvii., p. 272, 1894.

³ Weber. Hyg. Rund., p. 688, 1901.

Sidler. Arch. f. Hyg., xlvi., p. 327, 1903.

The process of boiling milk in the home for keeping it or using it as an ordinary article of diet is fairly common. It is more common in Europe than in America, and in the larger cities in Europe most of the milk which is directly consumed is first boiled. With those in our largest cities who do not have ice chests the custom of boiling the milk is also common, this being done for the purpose of keeping it longer. Such sterilization or boiling certainly has some advantages. Sterilized milk will never distribute any of the specific diseases above referred to, nor will it be the cause of diarrheal troubles due to the excessive growth of bacteria in milk. The inconvenience of boiling the milk, the development of the "boiled taste" that it produces, together with the fact that such milk is somewhat less digestible than the raw milk have prevented a wider adoption of this method. It is practically certain that the solution of the milk problem of our cities is not along the line of sterilizing or boiling.

PASTEURIZATION OF MILK

This procedure has recently become very widely known. The name was derived from Pasteur, who devised the method for treating wines and thus protecting them from various undesirable fermentations. The essence of the process is the heating of a liquid to a moderate temperature, sufficient to destroy many but not all of the bacteria, and insufficient to produce any very great chemical changes. The heating should be followed by rapid cooling. Pasteurization destroys a large part of the bacteria in milk, and this reduction in number greatly increases its keeping property.¹

The temperature at which pasteurization is performed varies considerably in different methods. In some cases a temperature as low as 140°, maintained for an hour or more, is adopted; in other cases the temperature of 160° is used for a short time, pos-

¹ Bitter. *Zeit. f. Hyg.*, viii., p. 240, 1890.

sibly 10 minutes. Sometimes a temperature slightly above the latter is adopted, but maintained for a shorter time, not more than a minute. Many machines and devices invented as pasteurizers produce quite different temperatures, and the effect, as would be expected, is very different with different methods. This must be borne in mind in trying to explain the effect of pasteurization on milk, and in determining its value.¹

The chief effects of pasteurizing are: 1. The maintenance of pasteurizing temperatures destroys a large part of the lactic bacteria. Inasmuch as these produce no spores they are quite easily killed, and as a consequence pasteurized milk will not ordinarily sour. When subsequently it shows signs of spoiling, as it will always do in time, the changes which appear are quite different from those in normal, unpasteurized milk.² 2. The temperatures adopted are generally sufficient to kill the germs of specific diseases, excepting tuberculosis. Typhoid fever, scarlet fever and diphtheria germs are all destroyed by comparatively low temperatures, and pasteurization is quite sufficient to kill them. Five minutes exposure to 155° will kill typhoid germs, and probably also those of scarlet fever and diphtheria. Whether pasteurization will destroy the tuberculosis bacillus will depend entirely upon its thoroughness. These organisms can be destroyed by a temperature of 160° maintained for 10 minutes.³ At lower temperatures a longer application of heat is necessary. At a temperature of 140° the bacillus will be destroyed,⁴ if the temperature is maintained for a couple of hours under conditions which prevent the formation of a scum on the surface of the "milk."⁵ Hence, the efficiency of pas-

¹ Freeman. Arch. f. Pediatrics, 1896.

Rogers. Bul. 73, Bu. An. Ind., 1905.

² Severin and Budinoff. Cent. f. Bact., II., xiv., p. 463, 1902.

³ Bang. Rev. Gen. d'Lait, I., p. 425, 1902.

Barthel and Stenstrom. Cent. f. Bact., I., xxx., p. 429, 1901.

⁴ Smith. Jour. Exper. Med., p. 217, 1899.

⁵ Hesse. Zeit. f. Hyg., xxxiv., p. 346, 1900.

Russell and Hastings. Rev. Gen. d'Lait, III., p. 34, 1903.

Russell and Hastings. Ann. Rep. Wis. Exper. Sta., 1904.

teurization in destroying the tuberculosis bacillus will depend upon the temperature and the length of the process. It is quite certain, however, that pasteurization as it is now frequently adopted in our cities, which means only a momentary heating of the milk at a temperature of about 160° , is not sufficient to destroy the tuberculosis bacillus.

The effect of pasteurization upon the bacteria that are probably associated with diarrheal diseases is as yet unknown, inasmuch as we do not know what they are. If, as it is believed, these troubles are due to the very great growth of some common bacteria, the use of pasteurization will be efficient in removing part of the danger of such troubles by destroying most of the germs. Practical experience has also shown that pasteurization is a useful procedure in the attempt to control the diarrheal troubles during hot weather.

It is a matter of great importance to know whether pasteurization of milk has any effect upon its digestibility or ease of assimilation. The heat applied to it is not sufficient to produce the chemical changes which occur in milk when it is boiled or sterilized, or the taste which develops in boiled milk, nor do the albuminoids of the milk coagulate as they do when it is boiled. Laboratory experiments have seemed to show that the digestibility of the milk is not impaired. There has been a general feeling in the past that while sterilization injures the digestibility of the milk, pasteurization does not do so. Recently, however, it has been claimed that pasteurization does impair the value of the milk, though laboratory experiments do not show it. It is insisted that its constant use has, in some cases, seemed to result in a certain type of disease, called rickets, that has been attributed to improper assimilation. The instances of this character are few and, as yet, somewhat uncertain. They have, however, produced in recent years something of a prejudice against the use of pasteurized milk. They are too rare as yet to constitute any valid argument against pasteurization.¹

¹ Doane and Price. Bul. 77, Md. Exper. Sta., 1901.

The efficiency of pasteurization differs much with the method adopted. When well done it may reduce the bacteria very greatly, for example, from 3,000,000 per c.c. to 10,000 per c.c., or even less. If less efficient the number at the close of the process would be perhaps some hundreds of thousands. The efficiency depends upon several factors. Dirty milk jars, or jars with dirty rubber rings, produce trouble, and other careless methods of handling decrease the value of pasteurization.¹ The most considerable factor affecting it, however, is the temperature, together with the length of exposure of the milk to the temperature. In general it is best to use lower temperatures for a longer time than higher ones for a shorter period, and it is doubtless best to pasteurize at a temperature no higher than 140°, if the process is maintained for a long enough period, *e.g.*, two hours; for this destroys all disease germs and produces the least change in the milk.² It is also important to do the heating in such a way as to avoid the formation of a scum on the surface of the milk, for such a scum makes the process less efficient in destroying disease bacteria. This can be done by some device to produce a constant agitation.

Pasteurization is sometimes adopted in a household on a small scale and sometimes in a central station for large quantities of milk. When adopted in the household it can be done by a very simple, easily devised apparatus. Figure 56 shows an apparatus which makes it possible to pasteurize the milk without the necessity of using a thermometer. The milk is placed in bottles, which are placed in a receptacle that has been partly filled with boiling water. The whole apparatus is then set aside to cool. The milk lowers the temperature of the water, and the hot water raises the temperature of the milk until they each reach the same temperature, after which the whole apparatus cools down. By proper adjustment of the size of the bottles and the

¹ Swellengrebel. *Cent. f. Bact.*, xii., p. 440, 1904.

² Jensen and Plattner. *Rev. Gen. d'Lait*, iv., p. 419, 1905.

amount of the water it is possible, after a little testing, to arrange the apparatus so that the milk will acquire just the temperature of pasteurization before it begins to cool. The use of such an apparatus is extremely simple, and after it is once adjusted can be successfully manipulated, even by those who do not understand the use of the thermometer. *Home pasteurization* has come to be a very common practice. The danger to children from tuberculosis and other troublesome diseases in the milk has not yet been guarded against by any public means. Hence, there is a growing tendency on the part of people of intelligence who are bringing up children on milk to pasteurize it. This plan is to be recommended in cases where the child must depend upon milk the source of which cannot be strictly relied upon. Perhaps such pasteurized milk is not as valuable a food as unpasteurized milk from absolutely reliable sources, but with the conditions in our cities, it is almost impossible to find milk that can be strictly relied upon to contain no dangerous disease germs. Under these circumstances pasteurizing is the only safe method by which a young child can safely be fed upon milk.

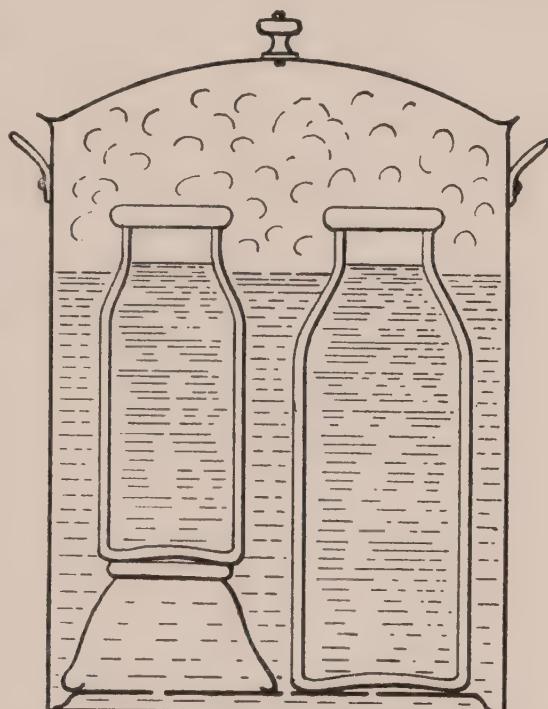


FIG. 56—SIMPLE IMPROVISED METHODS OF PASTEURIZING WITHOUT THE USE OF A THERMOMETER (RUSSELL)

PASTEURIZING ON A LARGE SCALE

In the last few years the plan of pasteurizing the milk on a large scale has come to be frequently adopted. This is done sometimes in creameries in connection with butter-making, and more frequently in some of our large cities for treatment of the general milk supply. In some countries the pasteurization of cream for butter-making is practically universal, but this is not true in America. In the pasteurization of the public milk supply the purpose has not been primarily to protect the public but to keep the milk from souring. Milk distributers have found it difficult to furnish milk that will keep without preservatives, but have learned that the application of heat enables them to do so. For this reason pasteurization has become widely adopted by large milk companies.

The pasteurization of milk on a large scale involves the use of special machinery, which it is not the purpose of this book to describe in detail. Two different principles are involved in the different pasteurizing machines.¹ One class is called *discontinuous pasteurizers*. (Fig. 57.) In these the milk is placed in a large receptacle and then heated, usually by steam running through coils of pipes of special devices. During the heating it is constantly agitated and is retained at the desired temperature for any length of time that may be wished; after which it is cooled by the running of cold water through the same pipes, or by some other device. It is then removed as pasteurized, and more milk takes its place. The second class is called *continuous pasteurizers*. Here the milk flows into a machine in a constant stream, and after passing through a series of conduits in which it is heated to the desired temperature, it is passed through others where it is once more cooled, and finally emerges from the machine in a continuous stream of cool, pasteurized milk. The efficiency of continuous pasteurizers depends naturally upon

¹ Weigmann. *Milchzg.*, p. 417, 1901.

the length of time that the milk remains in the machine. If the milk runs too rapidly, it will not be sufficiently heated; if it runs too slowly, it may be overheated; and the results, therefore, will vary widely.¹

Either the continuous or the discontinuous method may be efficient with proper care. The first is sometimes called *instantaneous pasteurization* since the actual heating of the milk

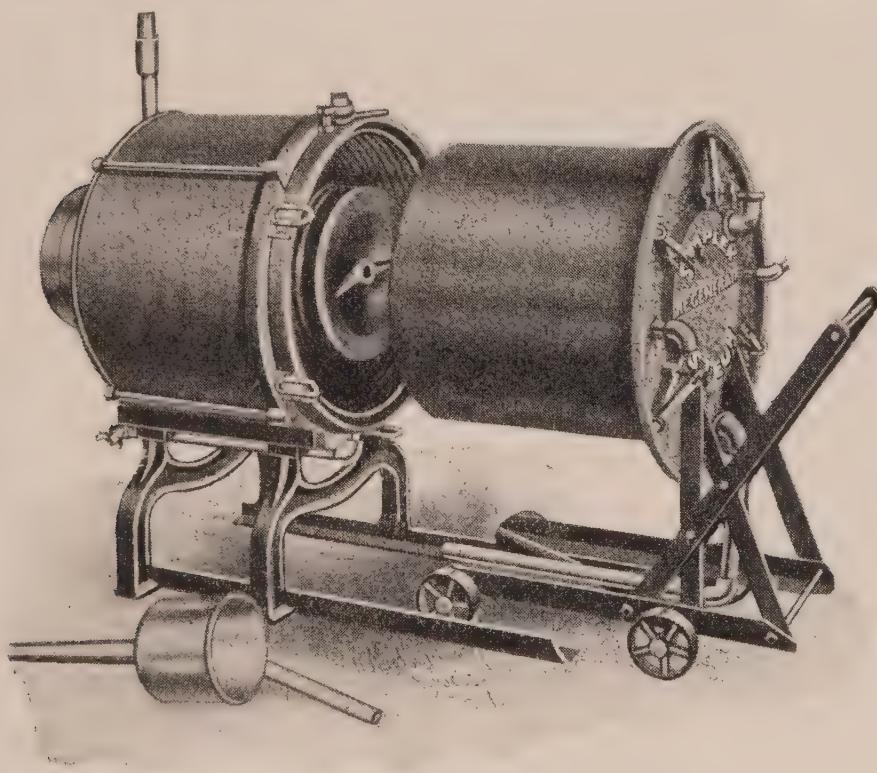


FIG. 57—PASTEURIZER OF THE DISCONTINUOUS TYPE
The milk is placed in the inner compartment. (D. H. Burrell & Co.)

is very brief, and the second is called *prolonged pasteurization*. It is obvious that the prolonged or discontinuous method will commonly be the more efficient. In the use of the continuous method it is a great temptation to hasten the process, thus reducing the time by running the current of milk too rapidly to allow a sufficient heat, and thus reducing the efficiency of the

¹ Russell. Ann. Rep. Wis. Exper. Sta., 1905.

process. Indeed, the milk is commonly heated no longer than one minute. While either method may thoroughly pasteurize the milk, there is a larger chance of error in the continuous pasteurizers, and practical study has shown that, as they are commonly used, the pasteurization by the continuous pasteurizer is less thorough than by the use of the discontinuous machines. As frequently used in the large milk supply companies, the pasteurization is of little value in improving the healthfulness of the milk. The milk is brought to the required temperature for an instant only, just sufficient to reduce the number of acid bacteria and thus increase the keeping quality of the milk, but not long enough to have much influence on the disease germs. There is, to-day, a move being made to bring this under proper inspection. If pasteurization can be properly controlled and placed under inspection so that its efficiency can be assured, it will be a very simple means of removing from the milk the danger of disease distribution.

In pasteurization the cooling of the milk after heating is of as much importance in preserving it as the original heating. The process does not kill all bacteria, and those remaining will subsequently grow rapidly unless the milk is cooled at once. From this it also follows that the milk should be used *as quickly as possible*, for while it will keep longer than unpasteurized milk the bacteria are growing in it all the while, and, since the acid bacteria are lacking, the putrefactive germs will have a greater chance to grow than usual. Old pasteurized milk is, therefore, worse than sour milk. It should be recognized also that only fresh milk should be pasteurized. After it has begun to sour the heat may curdle it, and it is never wise to use this treatment upon milk that contains more than 2 per cent. acid.

Pasteurization is sometimes applied to *cream* to enhance its keeping and enable it to find a market. The cream keeps well, but it loses some of its consistency. Such cream appears thinner than before treatment, and it will not whip as will ordinary

cream. Its consistency may be restored by adding a little of a material called "viscogen."¹ This is made by adding a strong solution of cane sugar to freshly slacked lime and allowing the mixture to stand until the upper part of the mixture is clear. This clear liquid is poured off and added to the cream in proportions of 1 part to 100 or 150 parts of cream. This restores the consistency to the cream, but since it is an addition of a foreign substance its use is illegal.

The effect of pasteurization upon the fat of milk proves a detriment in another way, since it interferes with the ready separation of the fat from milk. When the fat of normal milk is examined under the microscope the globules are found to be bunched together in little groups; but the fat globules of pasteurized milk are nearly separate from each other. This interferes with the cream rising as usual and there is no sharp line separating the cream and the milk after standing. This does not interfere with the use of the milk, but if it is desired to separate the cream from the milk it cannot be done so easily as with raw milk. In the feeding of infants it is sometimes desired to separate the top third for separate use, this third containing the most fat. Pasteurized milk cannot be readily treated in this way, so that the absence of a "cream line" is a real disadvantage.

TYPES OF MARKET MILK

The various suggestions for improving the quality of milk above given will, when followed out, produce a type of milk far different from that which has been common in our markets in past years. But it is impossible to carry out these various directions without expense. It is impossible to produce a high grade of milk for a low price. To obtain a type of milk that is being demanded by health officers will involve an expenditure which will evidently raise the price of milk. As soon as the public is

¹ Babcock and Russell. Bul. 44 Wis. Exper. Sta., 1896.

ready to pay the cost for the precautions suggested, the dairy-man will be willing to adopt them, but he cannot do so and furnish milk at the small price that has been paid for milk obtained under poorer, more careless conditions. As a result of this we find in our markets, to-day, several different types of milk.

Sanitary Dairies.—Near our large cities there have been appearing in the last decade sanitary dairies, in which all possible precautions are taken to produce milk of the highest grade. In these dairies the precautions given above are carefully followed out, and the greatest care is taken to produce a product above suspicion. The milk from these dairies frequently sells at twice the price of ordinary market milk, and even higher, 20 cents per quart being sometimes paid. Such milk is certainly more reliable and less likely to produce trouble than ordinary milk. Although there is already a large demand for such milk, these sanitary dairies do not meet the milk problem, for only a very small portion of the milk supply will ever be thus produced. The higher prices naturally prevent the people at large from purchasing it, and it is only the small number of persons, who have been willing to pay any price necessary to obtain a good product, who will receive the benefit. At the present time, therefore, sanitary dairies, while they have their place, do not reach the root of the milk problem.

That the precautions adopted in such places are efficient in reducing the number of bacteria is shown by the following figures obtained from the averages of a large number of samples of milk from four different sanitary dairies:

NUMBER OF BACTERIA IN MILK FROM SANITARY DAIRIES

Dairy No. 1 in winter	930	per c.c.
Dairy No. 1 in summer	1,050	" "
Dairy No. 2	1,400	" "
Dairy No. 3	2,000	" "
Dairy No. 4	280	" "

In Dairy No. 4 a separate milking room was used (Fig. 47). These numbers are very small, and the value of care in the dairy can be seen when the figures given above are compared with a similar series of averages from some common dairies. The best result in this second series was 60,000 per c.c., and the worst was 19,000,000 per c.c., in the milk, at the time it was delivered at the milk company's collecting station.

Certified Milk.—A somewhat different method of reaching the same end is by the production of certified milk. This is milk from dairies that have been inspected and passed upon by a certifying board as producing milk of satisfactory character. The Certifying Board is not always constituted in the same way, but it is usually in some way connected with health officials. The methods that are taken by these Boards to assure themselves that the milk is satisfactory are not always the same. They usually, however, involve an inspection of the dairies and a frequent chemical and bacteriological analysis of the milk. Dairies whose methods and whose milk meet a certain standard thus set by the Board receive a certificate for a certain length of time, which will be renewed later, if there are no reasons for the contrary. Such certified milk has certain advantages and certain disadvantages. The purchaser knows that he is obtaining milk that has been produced under at least fair conditions. He must pay a little more for it, although the price is always less than that of the milk of sanitary dairies, because less care and expense are involved in its production. But, on the other hand, no sample of milk, even if it is certified, can be guaranteed as free from disease germs or other contamination. The Certifying Board can certify only as to the general conditions of the dairy that produced the milk, never as to any particular sample of milk. It sometimes happens that certified milk is of no better character than the ordinary market milk, and sometimes it is not even so good. It is, however, on the average safer, and is becoming quite widely used.

Not a few farmers in the vicinity of our larger cities have recognized the possibility of furnishing certified milk and obtaining a slightly higher price for their product. It is not difficult for any farm to produce such changes in its dairy methods as will enable it to obtain the certificate of such a Board. The necessary precautions are not great or expensive, and inasmuch as a somewhat higher price may be obtained the plan is a very promising one and is being more or less adopted by up-to-date dairymen. Whether this plan of certifying milk in our larger cities is to develop into a more general method of procedure or whether some more radically different method of furnishing the city with milk is to be adopted cannot yet be stated.

The Production of Certified Milk.—While ordinary dairies are unable with the present price of milk to adopt all of the precautions suggested and advised above, there is no farm where certain changes cannot be made with so little expense as hardly to raise the price of production, but at the same time sufficient to produce a marked improvement in the milk. The production of certified milk does not necessarily involve great outlay, but may be done with fair success upon any good farm. It may be useful, therefore, to enumerate the simpler precautions which are most useful in improving the grade of the milk, the adoption of which will usually enable a dairyman to furnish certified milk. The following precautions are arranged according to their importance:

1. Veterinary inspection of the herd and testing all animals with tuberculin, followed by the rejection of all reacting animals.
2. Washing and sterilizing milk vessels and all dairy utensils. This should be done more thoroughly than is common on the ordinary farm.
3. The cleaning of the cows, clipping hair on the udders and hindquarters, the moistening of the udder and underside of the flanks before milking.

4. The use of a covered milk pail. The best are made of aluminum, since they are spun without seams. Milk should never be allowed to sour in them, for this will so infect them with bacteria that it will be very difficult to clean them. These pails should be used for no other purpose except to hold milk.
5. Covering the cans tightly when filled with milk.
6. Cleaning the cow stalls and barns. Anything short of clean stalls is repulsive.
7. Immediate cooling to as low a temperature as possible short of actual freezing.
8. Feeding after milking.

The other precautions which have been suggested above are of less striking effect. They may, perhaps, be placed in the following order:

Discarding fore-milk—clean hands and clothes for the milker—clean bedding for the cows—removal of milk from stall immediately after milking—ceiling of stables kept free from dust, preferably plastered.

There is no reason why any farm cannot adopt the precautions of better kept cows, cleaner stables, covered milk pails and more careful washing of the vessels, and every farm which will pay greater attention to these will obtain at once an improved grade of product. Indeed, many a dairyman by the simple adoption of these precautions, No. 1 to No. 8, will readily obtain the certificate of the Certifying Board.

Condensed Milk.—For many years condensed milk has been widely sold on the market. In condensing, a considerable portion of the water is removed by evaporation, and then for the purpose of preserving it a considerable percentage of cane sugar is often added. There is thus obtained a condensed product with a large amount of sugar, and one which will, when placed in sealed cans, keep for a long time. The large amount of sugar present very materially injures the value of the milk as a food, rendering it less digestible. For this reason, while condensed

milk is useful for some things, it can never be substituted for milk in an ordinary diet. It has undoubtedly a place in our food products, but it does not, and it cannot, take the place of milk for most purposes.

Unsweetened condensed milk is also upon the market. This material is sterilized by heat after being condensed to the desired volume. It is then sealed and it keeps simply because it is sterilized. It has the advantages and disadvantages of sterilized milk, and while an article of value for some purposes it adds little to the solution of the milk problem of cities.¹

The preservation of sweetened condensed milk is due to its great sugar content, which prevents the growth of bacteria. But sugar will not prevent the growth of yeasts. We have already seen (page 9) that yeasts can grow in sugar solutions. While bacteria do not usually spoil condensed milk it is sometimes found, to the misfortune of the manufacturer, that yeasts may ferment it. Immense losses have occurred in the condensed milk industry in the last few years by the growth of fermenting yeasts. Bacteria can usually be found in condensed milk also, but they do not develop. Hence, if yeasts do not produce trouble, the material keeps well for a long time.

Evaporated Cream.—This is a milk product which has been on the market for a few years, although not very widely used. It consists of milk with a varying amount of cream, evaporated to less than half its bulk and thoroughly sterilized by heat. It is then sealed in cans and will keep indefinitely. It has some of the disadvantages of sterilized milk, having much the same taste. Little is known of its digestibility, although laboratory tests² have shown that it is equally digestible with raw milk. Like condensed milk it has its place among food products, but it will not replace raw milk as an article of food.

¹ Kasdorf. Rev. Gen. d'Lait, I., p. 73, 1901.

² Mojonnier. Med. News, 1905.

Concentrated Milk.—A new method of treating milk has been recently devised which offers some exceptional advantages. The process is covered by patents, but it consists in the withdrawal of a considerable portion of the water from the skim milk, and the subsequent remixing of the product with the cream in proper proportion. In its preparation the use of heat by special devices is adopted, and the skim milk is treated for about two hours to a temperature of 140° . This reduces it to a thick mass which resembles cream. The cream, which has meantime been pasteurized, is now mixed with it in proper proportions, and the material is then known as *concentrated milk*. When the proper amount of water is subsequently mixed with this concentrated milk, 3 parts of water to 1 part of milk, it is restored to its original condition so closely as to be almost indistinguishable from fresh milk. It tastes the same, it curdles with rennet, cream rises upon it in much the same way as on fresh milk, and so far as experimental tests have determined, its digestibility is not in any degree impaired.

The advantages of concentrated milk are several. 1. Its treatment at a temperature of 140° for two hours absolutely destroys all disease germs, including the tuberculosis bacillus, so that there is no possibility of the product distributing infectious diseases. 2. The treatment reduces the number of bacteria so greatly that the chance of the milk being the cause of diarrheal troubles is practically removed. 3. The condensation of the milk increases the proportion of milk sugar. This produces a substance in which bacteria do not grow readily, although they grow rapidly enough if it is diluted with its normal amount of water. As a result concentrated milk may be kept, if cooled, for a very much longer period than any type of fresh milk. If kept at a temperature of below 50° , it will not only keep sweet for six to eight days, but at the end of this time the number of bacteria will be surprisingly few, for example: in a series of tests, after six days the milk contained less than

500,000 bacteria per c.c. After some days the bacteria will begin to develop and eventually become abundant, but during the first few days after its preparation it is possible to transport such material for long distances, and yet place it on sale in a city in a better condition, at least so far as bacteria are concerned, than most of the milk which now reaches the larger markets. 4. The expense of the transportation of such milk is very much reduced because of the decrease in bulk. 5. The concentrated milk is itself pasteurized, and because of its method of preparation is thoroughly pasteurized. The condensation of the milk is not possible by this method except by long-continued heating at, at least, a temperature of 140° , and this is sure to complete the pasteurization. This low temperature is not sufficient to give any cooked taste or apparently to produce any chemical changes except the evaporation of the water, in this respect being much better than pasteurization at a higher temperature; but it is sufficient, as shown by careful tests, to destroy the pathogenic bacteria of milk. For these reasons concentrated milk promises great aid in solving the milk problem of cities, since it offers at a low price milk that cannot be distinguished from ordinary milk, and yet can be guaranteed as free from pathogenic bacteria; and it will keep well. Attempts are being made at present to put this product upon the market in this country.

Milk Powder.—Many attempts to reduce milk to a powder have been made with more or less success.¹ The devices for this are several and need not concern us. In all cases where milk is reduced to a powder it is capable of being kept for a long time. Where the milk contains the cream, however, the reduction to the dry powder does not prevent its eventually becoming rancid, and powdered whole milk will not keep indefinitely. Powdered skim milk will keep without much trouble.

¹ Jensen. Rev. Gen. d'Lait, IV., 1905.

Huyge. Rev. Gen. d'Lait, III., p. 320, 1904.

These products have come to be well known in the last few years, and there are several of them, made by different processes, now on the market and widely used. None of them, however, really takes the place of fresh milk, because in no case has it been found possible to redissolve the powder again and get a milk that behaves like fresh milk. They are useful, especially in cooking, and in a variety of other ways, but it is doubtful if dried milk will ever replace the fresh milk of our cities.

Frozen Milk.—A method of meeting the milk problem by freezing the milk into cakes and pressing it in forms of bricks has been adopted in some localities in Europe.¹ The difficulty of its success has been in part that the freezing changes the character of the milk slightly, so that it is not always possible to melt the bricks of ice and obtain a good quality of milk. At all events this process has not been widely adopted and does not, at the present time, seem to promise a solution of the milk problem.

¹ Hittcher. *Milchztg.*, p. 292, 1902.
Bischoff. *Arch. f. Hyg.*, XLVII., p. 68.

CHAPTER VIII

PUBLIC PROBLEMS

A NUMBER of very vital questions have arisen in the last ten years concerning the relation of milk to the health of our communities, and the problem of the milk supply is becoming a very pressing one. Although this problem has not yet been solved, a number of factors have been fairly well settled. In the first place it may be stated beyond peradventure that cheap milk is always poor milk, and an improvement in the quality of the milk must mean an advance in price. We have just seen in the last chapter what a great amount of care is necessary to guard the milk from various sources of undesirable contamination and to preserve it after it has been produced. It is impossible to produce good milk at a low price, and the only hope for a better quality of fresh milk is that the public should be ready to pay a price sufficient to reimburse the producer for the extra care necessary to produce it.

THE CITY MILK SUPPLY

The condition in which milk reaches our larger cities varies greatly under different conditions. The bulk of it is brought in upon ice cars and consequently kept fairly cool; but the temperature varies greatly, with the different seasons of the year, with the amount of ice used and the care that can be given. It is the ice car that has made it possible to concentrate in our large cities the immense milk supply that is demanded. Since New York demands a quantity of milk so great as to require it to reach 400 miles for part of this supply, it is evident that only with the use of the ice car can this demand be met. It is also

evident that such milk cannot be furnished in the city in a very fresh condition. In fact, in some of our cities a large part of the milk is 48 hours old when distributed. Some of it may not be more than 24 hours old, but only a very small portion of it, from dairies close at hand, can be regarded as really fresh. It is not a matter of very great importance whether the milk is fresh or not, if it is only possible to keep it for the length of time necessary under conditions in which the bacteria will not develop too abundantly. If, therefore, it is kept sufficiently cool, milk from a distance may be, at the end of 48 hours, in a much better condition than milk from nearer dairies that has been brought into our cities, fresh, but that has not been properly cooled. The distance of transportation, therefore, is not necessarily a factor to render milk less wholesome, provided it is properly handled.

There has been a very great improvement in the conditions regulating the milk supply in the last 15 years. The milk is produced on farms with more care; farms in general are more cleanly, some of them very much more cleanly than formerly; and more attention is paid to the quality of the milk. This improvement in the dairy has resulted in an improvement in the milk, and at the present time our larger cities are supplied with a better grade of milk than before.

Curiously enough, these statements cannot be made in regard to the milk of the smaller communities, for these are sometimes at a disadvantage in comparison with the larger ones. In some states the larger cities have very stringent rules regulating the milk that can be sold, rules which exclude the milk from many of the poorer classes of dairies. But these same dairies, whose milk cannot be sold in a city, may send their product to the smaller towns and dispose of it without difficulty. This condition is doubtless only temporary, for laws will probably be adopted by which the smaller communities will be as well protected as the larger.

Need of Public Inspection.—The recognition of the close association between the purity of the milk supply and public health has given rise to a large amount of work on the part of health boards and others directed toward the control of the milk supply.

There is no food more easily adulterated than milk. Water can be added to it, and a variety of disinfectants and preservatives can be put in it without the consumer having any suspicion of it. For this reason the regulation of the milk supply has demanded a special set of rules quite different from those applicable to other foods. The individual is quite incapable of protecting himself, and he must, therefore, depend upon the expert knowledge of public officials, whom he must appoint for the purpose. The difficulty of the problem has rendered the methods of inspecting and controlling the public milk supply still more or less uncertain and tentative. Some of the rules and regulations that have been adopted and are now in force are undoubtedly of high importance, and some probably are mistakes which will be corrected as more information and better ideals prevail. The ends to be reached by a public control of the milk supply are three: 1. Chemical purity. 2. Freedom from specific disease germs. 3. Clean milk.

CHEMICAL PURITY

We are not concerned here with chemical problems, and the matter of chemical purity is mentioned here only because the same set of officials are likely to be called upon to consider chemical and bacteriological problems. The methods of testing milk may be found in books designed for that purpose. At this point it is only necessary to state that the chemical purity of milk is fairly well insured by public milk inspection. The different states have different laws as to the standard which a pure milk should reach, and they are in general so efficiently enforced that the public milk supply has a very fair grade of chemical purity.

FREEDOM FROM SPECIFIC DISEASE GERMS

The specific disease germs liable to be distributed by milk we have already noticed. There seems to be only one method by which milk can be guaranteed as free from disease germs. This method is by treating the milk to a heat sufficient for pasteurization. Inspection of the dairies will undoubtedly reduce the danger of disease germs getting into milk; but recognizing the wide occurrence of bovine tuberculosis, the difficulty of detecting it and the fact that the cows may have this disease in the udder unsuspected; recognizing, too, that cases of walking typhoid fever are possible, unsuspected by the patient or his friends, it will be seen that no kind of dairy inspection, no matter how rigid, can free the milk from the possibility of disease germs. No subsequent care or cooling of the milk can destroy them, if they have found entrance into the milk. Under these circumstances the sole practical method of assurance that the milk shall contain no specific disease germs is by adopting pasteurization or sterilization. All other methods of controlling the milk supply must be looked upon as a means of improving its general cleanliness and wholesomeness, and reducing the *chances* of the presence of the disease germs; but none of them can guarantee that the milk will be free from pathogenic bacteria. The danger of infecting the milk by germs of contagious diseases is so insidious that even in the best kept dairies and with the best intentions it will sometimes happen. Some of the sanitary dairies, kept under the highest condition of cleanliness, have been suspected of having distributed disease. Therefore, it follows that no methods are known by which a supply of fresh untreated milk can be positively guaranteed as free from disease germs. It must be recognized, however, that dairy inspection and the methods adopted for the improvement of the quality of the milk in general do greatly improve the milk by decreasing the chances of the distribution of the disease germs, but they do not entirely remove them.

So far as concerns the likelihood of milk being the cause of the diarrheal troubles, the matter stands differently. These appear to be associated with the *development* of bacteria in the milk, and any of the dairy methods already pointed out, which reduce the multiplication of bacteria, and which bring about a condition where the milk on sale in our markets shall contain a comparatively small number, will probably tend to reduce these dangers.

CLEAN MILK

The desirability of having clean milk needs no emphasis, but the methods by which this can be accomplished, and the methods by which it can be determined whether milk is clean, are not so simple. How is it possible to study miscellaneous samples of market milk and determine whether each was produced under conditions of proper cleanliness and with proper care? This question in one form or another has been asked over and over again; but up to the present time it has received no satisfactory answer. The method of determining this by actually estimating *the amount of dirt* in the milk, as indicated on page 297, is quite impractical. The accuracy of the dirt determination is so uncertain and the results in the hands of the different people show so much variance, that it has hardly been seriously suggested that the determination of the dirt in milk shall form a part of the municipal inspection. While such determination is of value, it is as yet impractical for the purpose of controlling or testing the public milk supply.

A method that has been gradually coming into adoption for this purpose is the determining of the *number of bacteria* in the milk. It is fairly easy to determine, with moderate accuracy, the number of bacteria, and if we assume that the value of the milk is in proportion to the smallness of the number of bacteria, it is fairly easy to reach conclusions as to its wholesomeness. Recognizing, as we have done, that bacteria-free milk is impos-

sible to obtain except by sterilizing, it follows that all market milk will contain some bacteria. Hence it is necessary, if this is to be our means of determining quality, to set a standard as to the number of bacteria that may be legitimately found in proper market milk. How large a number is consistent with the proper condition of the milk when it is placed upon the market?

PUBLIC CONTROL OF THE MILK SUPPLY

The growing recognition of the dangers in the milk supply and the inability of the individual to handle the problem for himself has led to a demand that the public health officials shall in some way guarantee the purity and healthfulness of the whole supply. The necessity for such a control is apparent, but it is not so evident how it can be brought about. Three quite different methods of reaching the end have been advocated: 1. *The use of standards.* 2. *Dairy inspection.* 3. *Compulsory pasteurization.*

THE USE OF STANDARDS

Milk standards may be arbitrarily fixed for chemical and bacteriological purity, and by constant analysis the dairies or dealers that fail to reach the standard may be determined. For *chemical purity* this works fairly well. For *bacterial purity* its value is not so clear, for even after a standard has been set, the enforcing of it is difficult and of doubtful wisdom.

Numerical Bacterial Standard.—The actual numbers of bacteria found are very great in the milk of large cities. In Chicago, a long series of tests showed it to range from 100,000 to 1,000,000 per c.c.; in New York, from 250,000 to 5,000,000; in Baltimore, from 1,500,000 to 4,500,000. The milk delivered to factories ranges from 5,000,000 in winter to 30,000,000 in summer. In small towns and cities the average is considerably lower than in the larger cities because of nearness to the source

of supply, 10,000 to 50,000 being frequently found; but even in these places many samples of milk show much larger numbers.¹

With such wide variations it clearly becomes difficult to determine upon any standard of numbers. At present, only in a few places has the attempt been made to establish such a standard. Boston has tried to enforce a standard of 500,000 per c.c., while it has been doubted whether New York could reach a standard of 1,000,000. Smaller standards than either are aimed at by some smaller cities, and for special grades of milk 30,000, or even 10,000, are sometimes demanded. Of course, it is not to be assumed that the milk that comes to New York is twice as dirty as that which reaches Boston, and hence it is evident at the outset that the number of bacteria alone is not a satisfactory means of determining the cleanliness of the milk.

The method of determining the number of bacteria in milk is fairly simple. Special collecting cases are provided, containing vials capable of being closed and cooled with ice. (Fig. 58.) These are filled with samples of milk to be tested, from the cars, delivering wagons or elsewhere. In collecting the samples it is necessary to stir thoroughly the contents of the can or jar which is to be sampled, and, of course, to determine and record with accuracy its source. Care in collecting the sample is of the utmost importance, for the milk at the bottom and the top of a can may be quite different. It is also customary to test the temperature of the milk at the same time and make a careful record. The samples thus collected are to be packed in ice and carried immediately to the laboratory. In the laboratory they should be immediately tested by ordinary methods, agar culture media being used in most cases at a dilution of 1,000 (see page 273), the plate being incubated at a tem-

¹ Jordan. Analyses of Chicago Market Milk. Chicago, 1904.

Park and Bebb. N. Y. University Bul. I., No. 2, 1901.

Harrison. Trans. of Canadian Inst., vii., p. 467, 1902-3.

Bergy. Univ. of Pa. Med. Bul., 1904.

perature of 98°. With this method the plates may be counted in 24 hours, and the number of bacteria in the original sample of milk determined.

The method of testing by the microscope, given on page 285, is adopted in some places with success, and gives a very much

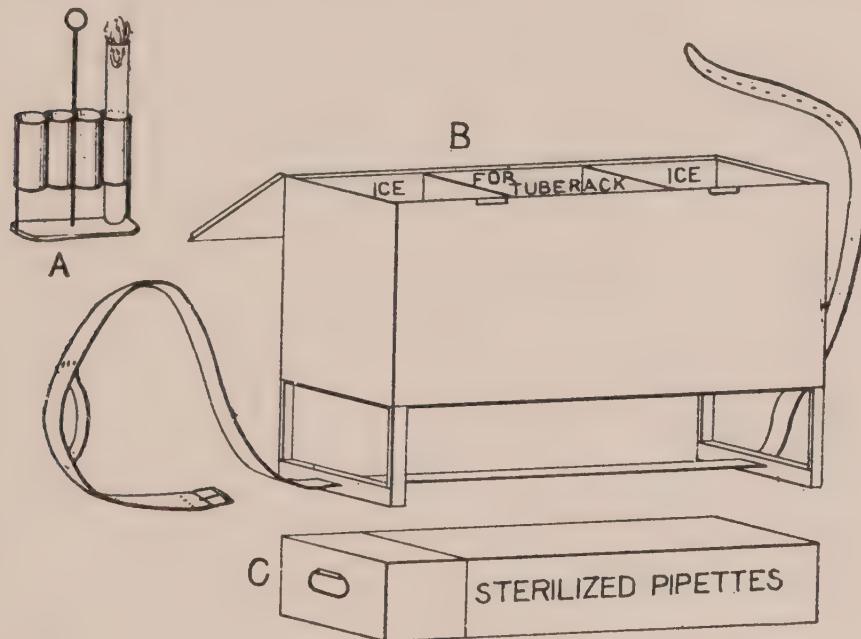


FIG. 58—COLLECTING CASE FOR MILK SAMPLES

The samples are removed from the cans with sterilized pipettes, and placed in tubes. These are placed in weighted racks (*A*) and put into the center compartment of the carrying case.

more rapid method of determining whether the sample of milk has such a small number of bacteria in it that it falls below a standard of 500,000 per c.c., or whether the number is so large as to run far beyond this. By the culture method at least 24 hours must elapse; by the microscopic method the results are fairly quick. In using the microscopic method it is desirable, if the microscope shows that the number of bacteria is probably in the vicinity of the 500,000 limit, to make agar plates at once in order that the test of the microscopic method may be con-

firmed by the test of the plating. If, however, the number of bacteria in a single microscopic field is not more than 20 to 30, the plating is unnecessary for determining that the number of bacteria is within a 500,000 per c.c. standard.

Significance of the Milk Standard.—When we consider the real significance of determining the number, we are forced to conclude that it is less than could be desired, and indeed it becomes questionable whether as now done its real value is worth its cost. The number of bacteria in any sample of milk depends upon three factors and not upon one. It is true that the original filth or cleanliness of the dairy, together with the care in producing the milk, is a factor contributing thereto, but the age of the milk and the temperature at which it is kept are, also, important. Indeed, as we have seen, the age and temperature are of more significance in determining the number of bacteria in milk than is the question of the original cleanly or filthy conditions. It is true that, even if fresh samples of milk are examined, the number of bacteria in the different samples will vary. Milk produced under good conditions may have as few as from 5,000 to 10,000 per c.c., while that produced under poor conditions may have from 100,000 to 200,000, or in some cases, perhaps, half a million per c.c. In other words, the cleanliness of the original conditions of milk production will determine variations in the number of bacteria in the milk *when strictly fresh*, ranging from a few thousand to a few hundred thousand. But, on the other hand, age and temperature run these few thousands up into millions, and it is not uncommon to find the number of bacteria rising as high as from 10,000,000 to 20,000,000 per c.c. Hence, the high numbers present in our city milk are dependent upon the age of the milk and the temperature at which it has been kept rather than upon the cleanliness of the conditions in which the milk is produced. Roughly speaking we may say that variations in number below 200,000 or 300,000 are due primarily to differences in the cleanliness

and care of the milk production; whereas variations in numbers from half a million upwards to several millions must be attributed to differences in the age and temperature of the milk. Furthermore, where we have very high numbers the reason lies practically always in a too warm temperature rather than any other factor. But these high numbers of bacteria usually consist of the harmless lactic acid type, and the fact that the numbers have run up to 20,000,000 *may* not detract in the slightest degree from the wholesomeness of the milk. High numbers of bacteria, therefore, do not necessarily mean unwholesome milk; while, on the other hand, milk may have a small number of bacteria and yet contain enough distinct specific disease germs to be decidedly dangerous.

One other feature connected with the numerical standard is to be noticed. The method used for determining the numbers by no means gives them all. As stated on page 175, the number is determined by diluting 1,000 times, plating upon agar and incubating at 98°. This really gives only a small part of the bacteria present. If the plates are made on gelatin and incubated at 70°, much higher numbers will be found. For example, a sample of milk diluted 1,000 times, plated on agar and incubated at 37°, gave 3,000,000 bacteria. The same milk diluted 10,000 times, plated at the same time on gelatin and incubated at 20°, gave 30,000,000. Usually there will not be found quite so large a difference, but there is always a smaller number found by the ordinarily adopted method than by using gelatin and incubating at lower temperatures. Hence, a numerical determination never gives the actual number of bacteria in the milk, but only of the numbers that grow upon agar and at temperatures of 98°.

Objections to a Numerical Standard.—For these reasons it becomes a serious question as to whether a milk standard is of much value. There are several objections which arise against its adoption.

1. The methods of analysis can never be of value in condemning any particular sample of milk, but only in giving condemnation of the dairy that produces the milk or the dealer who distributes it. It usually requires 24 hours before the number of bacteria can be determined, and before this the milk has, of course, been consumed. Even if the number of bacteria can be obtained two or three hours after the sample is received, it is quite too late to prevent the distribution and consumption of any particular sample of milk. All that can be done then is to condemn all of the milk from a certain source on the ground that one or two of the samples of milk have shown a number of bacteria above a certain standard. This is, of course, an unsatisfactory method of dealing with the question, but it is really the only possible one.

2. The use of a numerical standard is likely to be extremely unfair, condemning milk from some sources that should not be condemned and passing milk from other sources that should not be passed. A numerical standard, as we have just seen, is simply a somewhat indefinite gauge of filth, temperature and age combined. One dairy may have conditions of the most extreme slovenliness, and yet the proprietor is shrewd enough to cool the milk to a low temperature by the use of ice and thus check the development of bacteria. Under such conditions, though the milk is filthy and the conditions that surround his dairy are most unwholesome, the milk would readily pass the inspection, because the simple use of ice has prevented the bacteria from developing. The milk is likely to contain much that renders it unwholesome, but it will pass inspection. On the other hand, a dairy at a little greater distance from the city might be kept under conditions of scrupulous care in the milk production, and yet because of the use of less ice and thus less cooling, the milk before reaching the city would contain large numbers of lactic bacteria. Such milk would be condemned, and yet it would have nothing unwholesome in it. For it must

be remembered that it does not render milk unwholesome simply to have a considerable number of bacteria in it, provided these are of the ordinary lactic acid type. The result is that the milk of the well kept dairy may be condemned, while the milk of the slovenly kept dairy may pass inspection, simply because in one case a cooler temperature has been used, in spite of the fact that the cooler temperature has not rendered the milk more wholesome. This evidently makes a numerical standard unfair to the dairyman.

3. A numerical standard can under no conditions insure the wholesomeness of milk. The wholesomeness of milk is dependent upon the question as to whether it contains the types of bacteria that are liable to produce trouble and not at all upon the simple question of the number of bacteria present. A numerical standard primarily aims at the question of temperature and not at the question of cleanliness. Under these conditions it is evident that no sample of milk that falls under a numerical standard can be guaranteed as wholesome and none that pass beyond it can be condemned as unwholesome.

4. The enforcing of a numerical standard is very difficult. Where it has been attempted the success of enforcing it has been questionable. It is doubtful whether at the present time it is possible by any legal measure to enforce a rule which would require a numerical bacteria standard of milk. The only way that it has been enforced is by certain communities having the power to determine what milk may and may not be sold on the market. Where this is done the milk inspector can refuse to allow the sale of milk that comes from dairies producing milk with high numbers of bacteria. This has been done in some places with some success.

Value of a Numerical Standard.—In spite of these conditions a numerical standard has certainly proved to be of value. Its value, however, is indirect rather than direct. Where the numerical standard has been made fairly small, it has been

practically impossible for the dairy to meet that standard under the old conditions. When a standard is adopted the dairyman is usually supplied with directions for improving the methods and producing a better milk. The application of these methods becomes more and more rigid as the dairyman finds that his milk is likely to be condemned or rejected, because it fails to reach the bacteria standard. There is no question that with the improving of the dairy conditions and care in the production of the milk there results an improvement in the grade and a greater wholesomeness of the milk; hence it is that the adoption of numerical standards has had a tendency to raise the quality of the milk furnished in the city. As fast as the dairyman can be taught to use better methods in dairies, so fast will the danger associated with this product be reduced and tend to disappear. Care means safety, and the extent of the care is the extent of the safety. Inasmuch as numerical standards do bring about greater carefulness on the part of the dairyman they have been and are of emphatic value. Practically it has been found that in cities where a numerical standard has been set and where an attempt has been made to enforce such a numerical standard as thoroughly as possible, there begins almost at once an improvement in the quality of the milk. The number of bacteria becomes smaller, and the death rate of children falls in proportion. Hence, a numerical standard is of value in raising the type of milk and even reduces the danger of its being the cause of either specific or general diseases.

Qualitative Analysis of Milk Bacteria.—It is evident that a very much better knowledge of market milk could be obtained if it were possible by some simple means to determine not only the number of bacteria but their kinds. Such a complete analysis is not possible by any means yet known. The detection of disease germs is, as yet, practically out of the question. But a bacteriological analysis may be made that gives much more information than the simple determination of numbers, since the

determination of the relative numbers of the different types of bacteria enables us to draw some useful conclusions as to the age and purity of the milk. Such an analysis is also very useful when the attempt is being made to locate the source of special troubles that a cheese factory may experience.

The method of making such a qualitative analysis is given on page 304. To make it of any value it is necessary to know how to interpret the results.¹ To do this intelligently requires considerable experience with the work, but a few general rules can be given.

The first thing to do is to estimate *in percentages* each of the types found. Then, in determining the meaning of the different percentages, the following facts should be considered: If the number of bacteria is very high, 5,000,000 to 20,000,000, the percentage of *Bact. lactis acidi* should range from 20 per cent. to 50 per cent. If the percentage is lower than this, it indicates that this large number of bacteria contains many suspicious organisms, and that the milk should be condemned. If the percentage is as high as 80 to 90 per cent., the milk may be regarded as unsuspicious, even though the number of bacteria mount up into many millions. If the original sample has only a few hundred thousands, little inference can be drawn as to the healthfulness of the milk from the percentage of types of bacteria. If, however, the number of *liquefiers* is high, it indicates excessive filth contamination, while, if these are few and the acid organisms numerous, it indicates a high quality of milk. *Large numbers of liquefiers are always an indication of undesirable filth contamination.* These specifications are not very definite, but they cannot be made more definite from the fact that at present a more accurate qualitative analysis appears to be impractical.

¹ Conn and Esten. Ann. Rep. Storrs Exper. Sta., 1902-3.

SIGNIFICANCE OF DIRECT MICROSCOPICAL STUDY

Other facts can be determined by microscopic study which give information of value concerning the milk besides the determination of the numbers of bacteria. It will commonly be found that the specimen prepared as described in experiment



FIG. 59—SEDIMENT FROM CENTRIFUGALIZED MILK AS IT APPEARS UNDER THE MICROSCOPE

The large cells are leucocytes; the smaller ones bacteria

No. 17 will show a number of rather large, irregular cells (Fig. 59). These represent cells from the cow and are commonly spoken of as leucocytes. If, however, the udder should have any affection by which pus is being discharged, the pus will also appear in the milk and appear identical with the leucocytes. Does the presence of leucocytes in milk, therefore, indicate pus formation in the udder? That it does not necessarily do so is indicated

by the fact that practically all milk shows some leucocytes. They are identical with blood cells, and some of them appear at all times to pass through the milk gland into the milk where they cannot be distinguished from pus cells. Their presence does not, therefore, mean the formation of pus. But if they are especially abundant, it is believed to indicate pus formation and to make the milk suspicious.

Different observers have attempted to give some estimate as to the number of these cells that may be found in pure milk so as to make a standard by which it may be judged. But the number given as a standard has varied with the method adopted

for detecting them. Stokes, using one method, concluded that milk should be condemned if it contained more than 12 leucocytes per field of the microscope when studied with a one-twelfth inch immersion lens.¹ Stewart's method modified by Slack (see page 285) gives slightly different results, and when this method is used it has been assumed that milk should be condemned if it contains 25 or more cells to the field, which is equivalent to about 100,000 per c.c.² Doane's method of determining the number of leucocytes is more accurate and uniform. As described in experiment 18, it gives much higher numbers.³ Doane regards 500,000 leucocytes per c.c. as sufficient to render the milk suspicious and 1,000,000 per c.c. as indicating inflammation of the udder.

None of the methods yet devised can be regarded as very accurate or satisfactory. Considerable variations are found in successive determinations of the number of leucocytes in the same specimen of milk, and there is certainly no knowledge at hand to-day which enables us to say how large a number is sufficient to suggest that the milk really contains pus. All that can be said is that if the number per field of the microscope is much beyond 25, or the number over 500,000 per c.c., the milk may be regarded with suspicion. When mixed market milk shows a large number of leucocytes, it probably indicates that some of the cows supplying the milk are suffering from inflammatory troubles of the udder, and if the milk of a single cow should show large numbers, it probably indicates inflammation. But our information is, to-day, admittedly too small to make the determination of much value, and as a means of municipal examination of milk the determination of the number of leucocytes is not very satisfactory.⁴

¹ Stokes. *Ann. Rep. of St. Bd. of H.*, Maryland, 1897, 1898 & 1901.

² Stewart. *Am. Medicine*, ix., p. 486.

Slack. *Jour. of Infec. Dis.*, p. 214, 1906.

³ Doane. *Bul. 102, Maryland Exper. Sta.*

Savage. *Jour. Hyg.*, p. 123, 1906.

⁴ Rullmann and Trommsdorff. *Arch. f. Hyg.*, LIX., p. 224, 1906.

The value of a microscopic study may be somewhat increased by a careful examination of the types of bacteria found, since the presence of inflammation is accompanied by Streptococci in the milk. But, as we have already seen, Streptococci are practically always present in milk, it being almost impossible to draw milk from the most healthy cow without these bacteria getting into the milk. The presence of Streptococci in milk can, therefore, not be considered as meaning much of anything. The ordinary Streptococci of milk, however, are commonly not found as long chains but as isolated spheres, or in pairs. The pus forming cocci frequently grow in long chains. If, therefore, in a microscopic study there appear many long chains of Streptococci, this again may render the milk suspicious.¹

In short, if in making a microscopic examination, as described in experiment No. 17, the number of bacteria per field appears to be more than from 40 to 50, or if the number of leucocytes is more than 50 per field, or if there appear many long chains of Streptococci, the milk must be regarded as suspicious, requiring further study, or, perhaps, an investigation of the dairy where it was produced.

DAIRY INSPECTION

During the last few years there has been an increasing recognition of the value of public dairy inspection. This plan, though first suggested some 12 years ago in this country was first adopted by some of the large dairy companies in Europe.² In more recent years its value has been recognized, and the practice has been slowly but surely extending until at the present time it has become quite common in certain sections of the country. Five years ago a dairy inspection was scarcely known in the United States, but some of the larger dairy companies began first to organize a system of inspection of the

¹ Brüning. *Exper. Sta. Rec.*, xvii., p. 496, 1905.

Kaiser. *Arch. f. Hyg.*, lvi., p. 51, 1906.

² Conn. *Ann. Rep. of Ct. Bd. of Agri.*, 1895.

dairies that furnished their milk, and later some of our cities have adopted a similar plan. At the present time the plan of inspection of dairies by public officials is becoming quite common and is rapidly increasing.¹

A dairy inspection simply means that the dairies furnishing milk are visited periodically by some persons who are interested in the supply from the consumers' end and are not especially interested in the dairies themselves. They are familiar enough with the proper conditions in a dairy to make such a visit intelligently and to determine whether the dairy is carried on under proper conditions. These inspectors are supposed to make their appearance without any special warning, so that they will find the dairy in its normal, ordinary conditions. They are supposed to examine into all its details of milk production and distribution, to make recommendations to the dairymen as to improvements, and to make report to some headquarters as to whether conditions in each dairy are suitable for warranting a continuation of the dairy as a source of milk supply.

In such a dairy inspection the inspector must naturally take into consideration all the factors which contribute to the wholesomeness and cleanliness of the milk. He should consider all the phases of the dairy which we have outlined in the last two chapters. A summary of the points which should be examined by him may, however, be useful. His attention should be given primarily to the following factors:

1. *General cleanliness in the barn and dairy.*
2. *The condition of the cows.* By this is meant (a) whether they have been tested with tuberculin and demonstrated to be free from tuberculosis; (b) whether there are any signs of dis-

¹ Bergy. Penn. Dept. of Agri., 1900.

Pearson. 17th Ann. Rep. Bu. An. Ind., 1900.

Goler. Charities, Aug. 5, 1905.

Goler. Scheme for the Sanitary Control of the Municipal Milk Supply. Rochester, 1905.

Goler. Am. Jour. of Nursing, 1903.

Goler. Am. Medicine, 1903.

ease in the cows, especially of udder disease; (c) whether they are kept in a condition of cleanliness or are allowed to become caked with manure; (d) whether they are given exercise; (e) the kind of food which is used; (f) the time of feeding relative to milking.

3. The *source of the dairy water*. The source of the water which is the cow's drink is a matter of far less importance than the water used in the dairy for washing the milk cans and other utensils. This source of supply should be beyond suspicion. In suspicious cases, samples of water should be collected and sent to a proper laboratory for analysis, to determine whether the water is fit to be used in the dairy.

4. The *condition of the barn*; whether it has plenty of light and air, whether the manure is removed frequently, and whether dirt and dust are allowed unduly in and around the cows' stalls.

5. The method of *disposing of the manure*; whether it is allowed to accumulate in the barn, under the barn or nearby, or whether it is frequently removed to a considerable distance.

6. The *method of milking*; whether the milkers are careless or slovenly in their work, thus encouraging the entrance of great quantities of filth into the milk or udder of the cow, or whether they are intelligent and cleanly in their actions so as to produce milk under the best conditions.

7. The *condition of the milker*. This should refer to (a) the matter of his health, inasmuch as all suspicious cases of infectious diseases should be rigorously excluded from the dairy; (b) cleanliness of the milker before going to the milking. That the milker should make a careful toilet and wear clean clothes has already been emphasized and needs to be carefully watched by the inspector.

8. The method of *washing and sterilizing* the milk jars, cans and other utensils.

9. The *treatment of the milk after milking*. This should include the question of aeration, attention being especially given

to devices used for cooling the milk to as low a point as possible.

10. *Bottling of the milk*, if this is done in the dairy, or the method of placing the milk in the cans if the milk is to be carried in this manner.

11. The method of *transporting the milk*. This should include the temperature of the transporting receptacles, the cleanliness of these receptacles, and the method of handling them, and whether they are washed before they are returned to the farmer.

Recognizing that there are many things to be taken into consideration, it becomes evident that it is impossible to formulate any *standard* of cleanliness, which the dairy must attain in order to pass inspection. At best much must depend upon the personality of the inspector, and it will be to a large extent the matter of his judgment whether any dairy is in proper condition, where an improvement can best be made, and when a dairy must be excluded from furnishing the public milk supply.

Difficulties of Dairy Inspection.—While a dairy inspection has its emphatic advantage, and while it is unquestionably growing in popularity, certain somewhat serious objections have been raised against it.

1. It is difficult, at least at present, to get trained men who can properly serve as inspectors. Since there are so many matters upon which they must pass judgment, and since the personal attitude of the inspector is of so much importance, it is manifestly desirable that none except those who thoroughly *understand dairies* should be employed as inspectors. It is hardly possible to give to an untrained individual instruction which will enable him to carry on a satisfactory inspection. Trained men, however, are hard to find, and while there is no difficulty in getting persons who will serve as inspectors for the sake of obtaining a salary, it is not so easy to obtain men who are satisfactory. If inspectors are not properly equipped

for carrying on this work, they are pretty apt to produce more trouble than good and will certainly produce constant friction.

2. The value of dairy inspection is slight unless such inspection is frequent. There is little advantage in an inspector visiting a dairy and, if he finds it in poor condition, making recommendations and a report, and then going away and not coming back for several months. The farmer is very apt to pay no attention to the directions given him. To be of any efficiency, therefore, the inspection should be frequent. Where there are large numbers of dairies contributing to a milk supply, it is necessary that there be a large number of inspectors, if the different dairies are to be visited with any degree of frequency. To do this will require the expenditure of an almost prohibitive sum of money. An inspector, to be of any value, must have a good salary, for a poorly paid man is worse than useless. Considering the thousands of dairies furnishing milk to large cities, all of which must be inspected, it becomes a question whether a public inspection does not involve more expense than can be properly met. This, at all events, is one of the obstacles to an introduction of dairy inspection. Whether the money thus spent would not be one of the very best investments is another question. At all events, in the early stages of this attempt at introducing milk inspectors, these difficulties are prominent.

3. The enforcement of the rulings of the dairy inspectors has practical difficulty. It is one thing to have an inspector tell the dairyman to clean up his barn and make various improvements; it is quite another thing to get him to do it. Dairy inspection was first adopted by milk companies, and they have had very little difficulty in enforcing their regulations by a very simple means. These companies, in many cases at least, pay for their milk a trifle higher price than the ordinary price, and in such cases they can very easily refuse to receive milk from any dairy that does not reach the standard which they themselves set. Such a method of enforcement is very efficient. In

some of our cities a somewhat similar method can be and has been adopted. Cities have usually the power of making regulations concerning the food products that are sold in the market, and thus of regulating the sale of milk. The cities can put into the hands of a health board, or any other commission, the right to determine what milk shall or shall not be sold in a city. This commission can then take the ground that milk from dairies which their inspectors condemn cannot be sold in the city. This may effectually prevent the sale of such milk in the city, and, if properly carried out, will bring a strong influence to bear upon the dairyman. Another method of enforcing the instructions of the inspector might be by public statute. This is the most difficult and unpromising of all. The difficulty of enforcing laws is well known, and it is doubtful whether the punishing of a dairy because it has not reached a certain flexible standard of cleanliness would be possible.

There is one method by which an enforcement of dairy inspection might be reached, and that is by statutes which put into the hands of proper health officers everywhere the right to give licenses to the milk distributors in the communities or to withdraw them. If every milk distributor, in small towns as well as in large, requires a license to sell milk, then the officials would have the power of enforcing upon the dairyman the demand that the dairy and the milk should be kept in proper condition, and that the suggestions of the dairy inspectors should be followed. Such licenses are required in some places, especially in larger cities, but there is no such general rule at the present time. No other general method seems to be possible by which instructions of dairy inspectors can be enforced.

Inspector and Farmer.—The relation of the inspector and the farmer should be one of the most friendly nature, and it should be the purpose of each to help the other. It is no advantage to the farmer to deceive the inspector, nor is it any advantage to the inspector to be so rigid and severe in his condemnation as

to make it impossible for the farmer profitably to carry on his business. The purpose of the inspection is not to drive the dairyman out of business, but to bring about an improvement of the conditions under which the milk is produced. Any farmer would prefer to furnish good milk rather than poor milk. It is eminently desirable that the farmer and the milk inspector should, therefore, work together, and their attitude toward one another should be not that of hostility but of helpfulness. Of course, the extent to which this attitude will be developed will depend largely upon the individuals. Some inspectors will never take a friendly attitude to the farmer, and some farmers will always look upon the inspector as his enemy; but the friendly relation should be the aim of both the dairyman and the inspector.

Withal it must be recognized by dairy inspectors and all public officials that the application of proper dairy methods in the dairy depends wholly upon the readiness of the public to pay the price. *No amount of law will force the dairyman to adopt methods that he cannot afford at the price paid for his milk.* To obtain a quality of milk that is beyond suspicion requires the raising of the price *paid to the producer* above that which has been common in the last few years. It will always be possible, doubtless, to purchase good milk and poor milk. One cannot purchase silk for six cents a yard, but he can purchase a poor grade of cotton cloth. So we could not expect to buy first class milk which is beyond suspicion for a sum of money that is insufficient to reimburse the dairyman who employs satisfactory methods. The whole problem is one of price. The attitude of the public toward the producer should be not one of hostility but one of mutual assistance. The public ought to agree to pay willingly for whatever safeguards it demands for its milk, and as soon as this is done so soon will the dairyman be ready to adopt these safeguards. Rules and regulations will even then be necessary, but only to guide, not to force the producer.

COMPULSORY PASTEURIZATION

It has been evident from the facts already considered that it is extremely difficult, probably impossible, to put safeguards around the milk sufficient to guarantee positively its wholesomeness. But this can be done by the simple plan of compulsory pasteurization. It is impossible to guarantee that the milk that comes to our cities contains no harmful bacteria, but it is perfectly possible to destroy them by heat after the milk reaches the city, and thus guarantee the milk that is sold to the consumer. Should the officials who have power in the matter insist upon pasteurization of all milk offered for public consumption? Over this question there is a growing agitation.¹

Compulsory pasteurization has been adopted in some of the countries in northern Europe for all the milk products that reach the creameries, cheese factories, etc., but the suggestion of adopting it for the ordinary milk supply is new. In its favor is urged the fact that only thus can the public be positively protected against tuberculosis and other diseases distributed by milk. No dairy inspection, however much money is put into it, can give such a guarantee. Dairymen cannot all be made careful and cleanly, and even if they were, there would still be the danger from tuberculosis and walking typhoid. Pasteurization alone can insure freedom from disease germs. On the other hand, it is urged that although pasteurization is desirable and useful, it should be left to the individual to determine whether he wishes to adopt it. To be of value it must be thorough. We have noticed how inefficient the process may be when improperly carried out, and hence to be of real value it must be under public control, and under the inspection of the proper authorities. This would involve compulsory pasteurization, which would make it ordinarily impossible for the individual to obtain unpasteurized milk, if he wished to do so.

¹ Brown. Arch. of Pediatrics, p. 251, 1903.

It is claimed that pasteurization slightly reduces the digestibility of milk and hence should not be compulsory.

It is also urged that pasteurization will set a premium upon carelessness and render nugatory all attempts to improve the conditions in the dairies. If the dairyman knows that the bacteria which get into his milk will be killed by heat before the milk reaches the consumer, he will inevitably take less pains to keep them out, and consequently the dairies will fall back again into the condition of slovenliness from which they have been slowly emerging. Such a result is certainly not to be desired, and this is a valid argument against compulsory pasteurization, although it may be a question whether it is not better to have dirty milk rendered safe by pasteurization than clean milk that may contain tuberculosis and occasionally typhoid fever bacilli.

For these reasons the question of the wisdom of compulsory pasteurization is still unanswered. As yet, it is not anywhere adopted as a public measure, although not a few milk companies have adopted it as a means of protecting their milk from souring, frequently not informing their patrons of the fact. Compulsory public pasteurization is being strongly urged in some places, and it may possibly be the coming method of procedure.

CHAPTER IX

BACTERIA AND BUTTER-MAKING

IT IS evident that cream and butter will contain bacteria. They are present in the milk, they will be sure to be in cream when separated from the milk, and in the period that precedes butter-making they develop in great numbers. They are always present in cream whether sweet or sour, always present in butter whether fresh or stale. In butter-making it seems to be, at least according to present demands, absolutely necessary that the dairyman should have at his command large numbers of certain kinds of bacteria. The processes in butter-making are planned to insure the presence of the proper kinds and to stimulate their development rather than to check their growth. Indeed, in some cases, butter-makers have found it difficult to make satisfactory butter, because of too rigid attempts to exclude bacteria from their milk, and hence from their cream. For the butter-maker, therefore, bacteria are friends rather than foes.

CREAM RIPENING

The relation of bacteria to butter-making is associated with the phenomenon of cream ripening. The origin of the ripening of the cream is due to the early conditions of dairying when the butter was made wholly in small farms, and it was rarely possible to churn oftener than once in two or three days. It was necessary on such farms to allow the cream to accumulate until a sufficient quantity was collected to make good churning. During the period in which the cream was collecting it was sure to undergo the changes which have subsequently been spoken

of as ripening. When later the making of butter was concentrated into creameries, time for accumulating the cream was unnecessary, but the creameries soon found it necessary to keep their cream for a time before churning it in order that it should assume a condition similar to that of the soured cream on the isolated farm. From this came the process of ripening cream, which has been well-nigh universal.

The purposes of ripening the cream are several. 1. It is thought that the given quantity of cream will yield a *larger amount* of butter after ripening than when churned sweet. 2. It is certain, also, that cream will *churn more readily* when ripened than when sweet, although this does not apply to cream obtained by the separator, but rather to cream obtained by the gravity method. 3. Butter from well-ripened cream is thought to *keep better*. 4. But the primary reason for the ripening of the cream is in the *development of the flavor* to the butter. Sweet cream may be churned and butter obtained from it, but it will not have the ordinary butter flavor, showing rather the milder characteristic taste of sweet cream. This is the objection to the butter extractors—machines that separate the cream from the milk and churn it into butter in one process—since the fact that the cream was not ripened prevents the butter from acquiring the desired flavor. Doubtless the changes that occur in the cream during the period develop in it the flavors characteristic of butter.

Sweet cream butter, however, is in considerable demand in some places. In Europe there is a general tendency to prefer a milder flavor in butter than is preferred in America. An American traveling in Europe is apt to find the butter tasteless, while a European is apt to complain of American butter as too strong. In many parts of Europe a very slight ripening of the cream, or no ripening at all, is adopted, and the butter thus obtained has very little flavor. Frequently the butter is not salted and is thus more delicate than the highly flavored butter

of America. Some people are very fond of such mild butter. A demand for sweet cream butter has developed in America; but at the present time this demand is small and forms only a very small part of our butter product. There has been a change in public taste concerning butter during the last 25 years. At present the demand is for a milder flavored butter than a generation or so ago, and a strong, high flavored butter will not find, to-day, as ready a sale as it formerly would. Butter that would formerly have sold readily as first grade would now be regarded as too strong and ranked as second or third quality. No doubt there has been, even in the United States, a general tendency for a milder type of butter. Whether this tendency will develop further until our markets demand a large amount of sweet cream butter cannot, of course, be predicted.

The Cause of Cream Ripening.—The ripening of cream is a phenomenon of bacteria growth. The bacteria which are in the cream find it an excellent medium for food, and if kept at a fairly warm temperature during the ripening period, their development is quite rapid. For the 12 to 24 hours of ripening the bacteria multiply, and by the time the cream is ripened and ready to be churned they are present in prodigious numbers. Many analyses of ripened cream have been made, disclosing the fact that whereas in the sweet cream bacteria may be 2,000,000 to 3,000,000 per c.c., in the same cream when ready to churn there may be about 500,000,000 per c.c. The numbers at the time of ripening, however, vary widely, being sometimes as low as 200,000,000, or even lower, and sometimes as high as 2,000,000,000 per c.c. There does not seem to be any especially close connection between the completeness of the ripening and the number of bacteria. In some thoroughly ripened cream samples there may be two or three times as many bacteria as in others, and yet no difference in the completeness of the ripening can be seen.

The growth of bacteria in the cream produces **critical**

changes which considerably modify its nature.¹ The lactic acid bacteria always develop lactic acid, and the cream becomes sour; but there are other changes also. We do not yet know what all these changes are nor to what extent they contribute to the ripening phenomenon. That the other changes have something to do with the production of the flavor in butter is evident from the fact that a butter flavor cannot be produced in the cream by adding lactic acid to it, and if the ripening were wholly the result of the souring, the addition of lactic acid should produce the same result as normal ripening.

Growth of Bacteria During the Ripening.—The study of the growth of bacteria during the ripening of cream is quite suggestive and instructive. At the outset the cream is quite certain to contain numerous kinds of bacteria, differing much, of course, in different samples. In the composite cream of a creamery the varieties will be very numerous, while in cream of a private dairy they may be less numerous. During the ripening the cream is commonly kept between 60° and 70°, at which temperature many bacteria develop rapidly, but not all kinds with equal vigor. During the first few hours of the ripening there is found to be a general increase in the number of bacteria of nearly all kinds that were present in the original cream, so that after six or eight hours there will almost universally be found higher numbers of all species of bacteria than were found at first. During this time, however, the lactic acid bacteria, especially of the *Bact. lactis acidi* type, have been increasing more rapidly than the others.² In the very fresh cream these bacteria may have been, and commonly are, com-

¹ Conn. Ann. Rep. Storrs Exper. Sta., 1890, pp. 91, 93 and 94.

Conn. Cent. f. Bact., II., ii., p. 409, 1896. III, p. 177, 1897.

Conn and Esten. Cent. f. Bact., II., vii., p. 743, 1901.

Conn and Esten. Ann. Rep. Storrs Exper. Sta., 1900, 1901.

Storch. Cent. f. Agr. Chem., 1891.

Haywood and McDonnell, Bul. 44 Penn. State Col. Exper. Sta., 1899.

² Conn. Bacteria in Milk and Its Products. P. Blakistons Son, 1903.

Conn and Esten. Ann. Rep. Storrs Exper. Sta., 1901.

paratively few in numbers, forming sometimes not more than 1 to 2 per cent.; but their percentage rises rapidly until they come to form an appreciable portion of the whole. After several hours, the time varying with different specimens, the acid bacteria constitute a large percentage of the whole. From this time, after they form perhaps 50 per cent. of all of the bacteria present, the other species begin to be injuriously affected by the growth of the lactic bacteria, probably by the acid produced. The acid forming germs still continue to increase in numbers, while the others cease to grow so rapidly. After a time they begin to diminish and may finally largely or wholly disappear. The result of this is that during the last stages of the ripening there may be present in the cream nothing but acid bacteria, which sour the cream and produce the final changes in the ripening.

It will thus be seen that the ripening of the cream may be divided into two stages: In the first the growth of the miscellaneous species of bacteria continues, and all types may become more or less abundant. In the second the acid-forming germs take possession of the cream and gradually force the others into the background and, finally, may crowd them out entirely. Both of these stages doubtless contribute to the final product. Without the proper lactic organisms it is impossible to get the proper flavored butter. But butter made from pasteurized cream and ripened by pure cultures of lactic acid bacteria does not develop so much flavor as that in which the original bacteria are allowed to grow with the acid germs. Hence, it is probable that the development of the miscellaneous bacteria in the first phase of the ripening has not a little to do with the final butter flavors. Concerning the matter, however, nothing is positively known, the problems being too complex to have admitted of any solution at the present time.

THE NEED OF CONTROLLING THE RIPENING

The butter-maker needs bacteria, but he wants *the right kind*. The character of the butter obtained from the cream is closely dependent upon the kind of bacteria which develop in it. Many experiments have been performed of making butter from cream ripened with different species of bacteria, which have shown that very different results come from the use of different bacteria.¹ Some species produce unpleasant taints, bad tastes, bitter tastes and other undesirable results; many species have been found to be quite negative in their action on the cream and resulting butter. Other species, again, develop pleasant tastes and odors, and are advantageous and useful in the cream ripening. It chances, fortunately, that the latter is true of most of the lactic acid bacteria. Since in the ordinary bacterial growth in cream the lactic bacteria in the end finally get the upper hand and grow at the expense of all of the others, it commonly happens that the ripening produces a good flavor, and a satisfactory butter is obtained. If this were not true, butter-makers would never have adopted this process of cream ripening, for, of course, ripening has been adopted because it has been found to be commonly successful in giving a desirable product. Unfortunately, however, the favorable species of lactic bacteria do not always get the upper hand in the cream ripening. It sometimes happens that there are present in cream large numbers of vigorous bacteria just as capable of rapid growth as are the desirable lactic acid germs. In these cases the unusual bacteria may develop abundantly and produce a variety of uncommon changes in the cream, with the result of giving an undesirable flavor to the butter. Such a phenomenon explains the occasional appearance of bad tasting butter.

¹ Conn. Ann. Rep. Storrs Exper. Sta., 1894.

Eckles, Cent. f. Bact., II., iv., p. 730, 1898.

When butter was made upon the small farm the occasional appearance of an improper ripening was a matter of no very great importance. The butter is rarely so bad that it cannot be eaten, and the farmer who made his own butter did not particularly trouble himself if, for a few days, or even for a few weeks, his butter had a stronger taste than usual. When, however, butter came to be made in creameries, the necessity for producing a uniform product was greater. When a creamery is producing hundreds of pounds of butter per day, and when the market price will depend upon the character of the butter, it is a matter of more importance to obtain a desirable flavor. A good flavored butter will yield a high price, and poorly flavored butter will inevitably yield a lower price. Under these circumstances, the question of financial failure or success may hinge upon flavor, and this in itself depends upon the ripening of the cream. Hence, in recent years all creameries have come to recognize the vital importance of a proper control over the ripening. Inasmuch as this ripening is a bacterial problem it follows that the success of a creamery is, to a large extent, dependent upon the control of its bacteria.

Flavor and Aroma.—The general character of the butter is determined by both flavor and aroma. The term flavor refers to the taste of the butter, whereas the term aroma refers rather to the smell. These two factors do not appear to be the same, and in some cases, at least, they have a different origin. Some bacteria give rise to a desirable flavor in butter but do not give rise to an aroma; others give rise to an aroma without much flavor; and different species still produce neither acid, flavor nor aroma.¹ It is not understood from what materials the flavors and aromas are produced.² That they are not caused by the acid in the cream alone has already been mentioned. They are not due primarily to the fat, as is shown by the fact that they will appear in ripened skim milk.³ That they are

¹ Conn. Ann. Rep. Storrs Exper. Sta. 1894.

² Weigmann. Milchztg., p. 193, 1897.

³ Mickels. 22d Ann. Rep. Wis. Exper. Sta., 1905.

caused by certain bacterial decomposition products is evident; but whether they are the result of the action of bacteria upon the casein or upon the albumen, or the milk sugar, or the fat, is not yet settled. The question is a complex one, but the probability is that flavors and aromas are not due, in ordinary butter, to any one kind of bacterium or to any one kind of decomposition, but that quite a variety of processes may contribute thereto. It is certain, however, that the production of both flavor and aroma is more or less independent of the production of acid. By this it is not meant that the bacteria producing acid may not also produce flavors and aromas, but that the two phenomena of acid production and flavor production are not identical. What evidence we have to-day indicates that the production of flavor and aroma is not primarily associated with the common lactic acid bacteria, and must be attributed in a measure to certain other types of organisms.¹

CONTROL OF CREAM RIPENING

If the quality of butter is so closely dependent upon the nature of the cream ripening, it is extremely desirable to control this phenomenon. In the earlier periods of farm butter-making no attempt was made to do this, and the result was a great irregularity in the butter. As the industry has become more concentrated greater stress has been laid upon the cream ripening, until at the present time the butter-makers have this process largely under control. Twenty years ago little attention was given to cream ripening; to-day it receives more care perhaps than any other phase of butter-making. The methods of regulating it are several.

1. Regulation of Temperature.—The temperature which is recommended for cream ripening has varied all the way from

¹ Grimm. *Cent. f. Bact.*, II., viii., p. 584, 1902.

Severin. *Cent. f. Bact.*, II., xi., p. 202, 1903.

Van der Leck. *Cent. f. Bact.*, II., xvii., p. 467, 1906.

50° to 75°. In earlier years, higher temperatures have been adopted. There are certain important reasons for the higher temperature. In a well-ripened cream there must be an abundance of lactic acid bacteria, and these do not grow well below 60°. Certain other species of bacteria, which produce a bitter taste, are more likely to develop at low temperatures than at high. If, therefore, cream is ripened at too low temperatures, the acid organisms do not grow so well, and there is a greater chance for the bitter bacteria to develop sufficiently to produce an undesirable flavor. On the other hand, at too high temperatures other types of bacteria, especially gas-producing forms, and others equally undesirable, are particularly favored. A temperature from 65° to 70° appears, on the whole, most likely to avoid both of the difficulties, and under ordinary circumstances the best results can be obtained by a temperature from 65° to 70°. In warmer weather it may be desirable to use a lower and in cold weather a somewhat higher temperature.

2. Duration of Ripening.—The duration of the ripening varies very much with the conditions. Sometimes cream when brought to a creamery is already nearly sour and has, therefore, become ripened even before the butter-maker receives it. In other cases, especially in winter, it will be not only very sweet, but will contain small numbers of bacteria, requiring a much longer ripening. Moreover, milk produced under good dairy conditions, clean and fairly free from bacteria, will ordinarily require longer ripening than milk that has been produced under less favorable conditions, and that consequently already contains bacteria in great numbers. The length of time will vary also with the temperature, being, of course, longer at lower temperatures. To determine when the cream is sufficiently ripened the butter-maker has two methods. One is the general appearance to his eye and taste, the other is by determining the acidity. The production of the acid may not be the whole of cream ripening, inasmuch as it has little to do with the flavor and

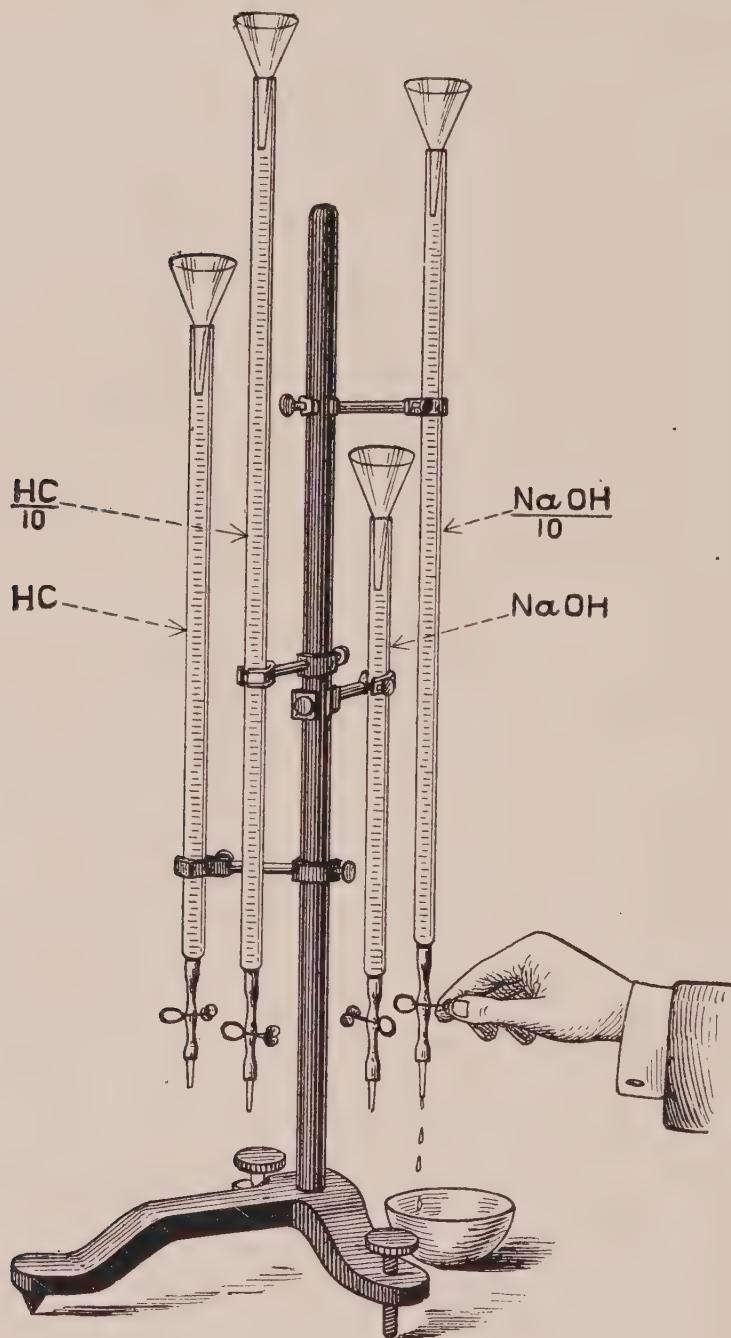


FIG. 60.—THE USE OF BURETTES FOR DETERMINING THE AMOUNT OF ACIDITY

aroma; but, as a rule, flavors develop parallel with the acidity, and if the acidity becomes too high the flavors become too strong. It is a fairly easy matter to determine the acidity of

cream, and as a practical procedure it has become common to determine the degree of ripening from its amount of acidity. The general practice of butter-makers is to ripen the cream until the acidity reaches about .5 to .65 per cent. of lactic acid. This is determined by the well-known methods given in other places and need not be repeated here (Fig. 60). If the cream is very rich, containing a higher per cent. of fat than usual, the ripening should not be continued to quite such a high per cent. of acidity. The reason for this is that the acidity is dependent upon the fermentation of milk sugar, and if the per cent. of fat is very high, there is a smaller amount of milk sugar than usual, and the acidity will not rise so fast; hence, the ripening becomes completed with a lower acidity than usual. In using the acid test as a method of determining the ripening of cream it must be remembered that this test only gives the grade of acidity, and while this is a gauge it is not the whole ripening.

3. Use of Starters.—The greatest change that has taken place in butter-making in recent years is the adoption of more and more careful methods of regulating cream ripening by the use of starters. Twenty years ago they were only occasionally used; to-day their use is well-nigh universal. A starter simply consists of a lot of cream or milk, which has become already soured, and is added to the mass of cream in the cream vat for the purpose of controlling the ripening process. It was first used by butter-makers in cold weather only. In the early days of creameries it was found that during the cold weather the cream frequently would not ripen quickly, and though left for the ordinary time in the cream vat, it was not ready to churn when desired. This was easily obviated if the proper amount of sour cream was added to it. This was called "starting" the ripening, and hence the name starter. Although used originally only to start the process, it has been found that starters have other advantages. By their use butter-making

can be much more accurately controlled. 1. The *flavor and aroma* of the butter resulting from the ripening may be to a large extent modified by the use of starters and rendered more uniform. 2. A *greater uniformity* in the product has been found possible by using a properly prepared starter. The matter of uniformity, especially in the handling of butter, is of much practical significance. It is of importance to the manufacturer that his cream should reach its proper stage for churning at practically the same time every day, otherwise the whole routine of the creamery is disturbed. By the use of starters of the proper type and used in the proper way, the ripening can be so controlled that the churning can take place at practically the same time every day. 3. The use of starters has frequently been found valuable in avoiding *undesirable taints and tastes* in the butter. These unpleasant flavors, which even in the best creameries occur occasionally, can frequently be checked or remedied by the use of a considerable quantity of a proper starter.

THE MAKING OF STARTERS

Home Starters.—Under this head is included a type of starter that can be manufactured in the ordinary dairy; for it consists of nothing more than a good type of sour milk or cream. In order to obtain this, it is only necessary to select several quarts of milk, preferably from a cow that gives a good quality. If a creamery wishes to make such a starter, it will select the milk from one of the better kept dairies among its patrons. This milk is then placed in a clean, sterilized pail, or can, covered to keep out the dust, and placed at a temperature of from 65° to 70°. After 24 to 48 hours the milk should show signs of souring, and after it has become decidedly sour, but *not yet curdled*, it is to be used as a starter. In the preparation of such a starter, evidently all that is needed is a fair quality of milk, clean pails and a proper temperature. It must be noted, how-

ever, that it requires some skill on the part of the butter-maker to know whether the starter that he thus obtains is of the best character, and whether it should be used or thrown away, and another obtained. Starters made in this way are not sure to be uniform, inasmuch as the different samples of milk may contain different types of bacteria, and experience is needed on the part of the butter-maker to know whether the starter thus obtained is satisfactory.

Starters from Commercial Cultures.—In the last few years there has been placed upon the market quite a variety of commercial cultures for the purpose of controlling butter-making. These are prepared by bacteriologists and consist of many types of bacteria that have been found by experiment to produce a satisfactory ripening. The bacteria have usually been isolated from ripening cream, and have been chosen from a large number of tested species which promise to produce a desired type of ripening and, consequently, the best grade of butter. These commercial cultures are commonly called *pure cultures*, which means that they consist of one species of bacteria only. We have already noted that the flavors, aromas and acidity are probably not produced by the same organisms. Probably no bacterium combines in itself the requisite powers of producing all three factors. Pure cultures, therefore, might not be expected to give all the qualities desired in a first class butter. To meet this difficulty some of these commercial cultures consist of a mixture of bacteria of two or more kinds, in order to produce a starter which will furnish at the same time flavor, acidity and aroma. These bacteria cultures, whether pure or mixed, are developed in a bacteriologist's laboratory until the organisms are in great abundance, and are prepared for market in various ways. In some cases they are mixed with some inert powder and sold in a dry form. In other cases they are sent in the form of a bouillon culture much like those described on page 295. Others again are cultivated in milk and sent out as milk cultures.

The question of the *purity* of the commercial cultures is of the greatest importance. Inasmuch as they consist of living organisms it is absolutely necessary that the cultures should contain the organisms desired and no others. It would be of no great value to add to a lot of cream a mass of bacteria, unless it contained the desired organisms and no others. During the first years that such cultures were put on the market they were very apt to be impure, and even at the present time they are not absolutely reliable. It is an extremely difficult matter to produce bacteria in quantity without their becoming contaminated with undesirable species; and in the commercial cultures it occasionally happens that unexpected and undesired species of bacteria or molds find their way into the cultures, and injure or ruin them. It also sometimes happens that the culture, though pure, may *lose its vigor*, for bacteria retain their greatest vigor only under the most favorable conditions. As better methods of cultivating bacteria are learned, the difficulties of impure cultures are disappearing, and the cultures are becoming more and more reliable each year. At the present time the commercial cultures can be fairly well depended upon. But even to-day the cultures put out by one of the most reliable firms occasionally contain species that are not wanted. Absolute purity and uniformity of the cultures have not yet been reached.

In preparing a starter from a commercial culture the dairyman must remember that he is dealing with living organisms and must handle it accordingly. It *must not be heated*, for this would kill the living organisms. It must be used, if possible, *while fresh*, for the bacteria in the culture begin some days after to lose their vigor, and eventually die. An old commercial culture, therefore, is useless. The culture must be treated in such a way as to give the bacteria the *greatest chance for developing*, since the growth of these organisms produces a starter. The cultures are usually sealed when they leave the bacteriologist's laboratory. Care should be taken, if a commercial article be

used, to see that *the seal of the package is unbroken*. If the seal is broken, there has been an opportunity for contaminating bacteria to enter. The culture, if not used immediately upon receipt, should be *kept in a cool, but not cold place*; for example, in a refrigerator, not on the ice but near it. At this temperature they will keep best. Lastly, in most cases, *the whole culture should be used at once*, and the dairyman should make no attempt to use half a culture and reserve the other half for a later date, especially if it be a liquid culture. It is almost impossible for him to remove half of such a culture from its package without so contaminating the other half that, if preserved for use at a later date, it is no longer reliable.

Preparation of a Commercial Culture.—To prepare a starter some plan must first be adopted to increase the number of bacteria, for the package bought in the market does not contain enough of these organisms to furnish a starter for any considerable quantity of cream. If only a small amount of starter is needed, for example, a quart for a few gallons of cream, the procedure is as follows:

A quart of skim-milk is placed in a glass jar and sterilized either by boiling, or better by pasteurizing, by submitting it to a heat of 180° for half an hour, stirring frequently to insure uniform heating. The milk is then cooled by placing it in cold water, and when it has reached a temperature of 80° the commercial culture, from a freshly opened package, is stirred in thoroughly; the whole is covered to keep off the dust, and placed at a temperature of about 65° . When the milk has become quite sour, but before it has curdled, it is ready to use as a starter. It is much better to use the starter before curdling takes place, for two reasons: 1. While the milk is still liquid it is easy to distribute a starter uniformly through the cream that is to be ripened, so that with a little stirring the whole mass of cream is inoculated with the bacteria. After the milk has curdled it is much more difficult to distribute it uniformly

through the cream. 2. The lactic acid bacteria which are especially desired in the cream appear to be most vigorous just before the souring. Before the milk has soured or after it has curdled, they do not seem to have so much power as they do just before curdling. Care should be taken never to allow the material to cool to a temperature much below 65°. If it does, undesirable bacteria are liable to grow, and the growth of the desired organisms is checked.

If a larger amount of starter is needed, for a creamery where there are hundreds of gallons to be ripened, the preparation of a starter must be continued farther. The starter is prepared in exactly the same way up to the last step. Meantime several gallons of cream or skim-milk have been pasteurized, the amount depending upon the size of the churning. This should be cooled to 80° at about the time when the starter prepared according to the last paragraph has become soured. This starter is then poured into the new lot of pasteurized milk or cream, the mixture is covered to keep out the dust, placed at a temperature of about 65°, and when it has become well soured, it is a starter of sufficient quantity to be used in a vat of hundreds of gallons of cream. This process is spoken of as a "building up process." Evidently the whole process is only a means of increasing the number of bacteria by growing the culture in a considerable amount of pasteurized milk.

The starter thus prepared is added to the cream in varying proportions, the larger the amount the quicker the ripening, a quick ripening being, however, generally undesirable.¹ Sometimes as much as 1 part of the starter to 10 parts of cream is used; in other cases a smaller amount is used and sometimes more. After the cream in the vat, into which the starter has been inoculated, has been ripened for the proper length of time and is ready to churn, a certain quantity of it is removed, placed in a clean can, and set aside to serve as a starter for the next

¹ Dean. Ont. Agr. Col. Exper. Sta., Farm. Rep., 1901.

day's churning. In this way some starter is reserved each day to be used in the cream collected for the day, and thus the original starter is carried on from churning to churning. After some days, however, it is necessary to resort once more to a new pure culture built up in the same way. The reason for this is that the miscellaneous bacteria in the cream may develop in larger numbers each day, until eventually the cream contains too many of the undesirable organisms. How long a starter may be carried from day to day before a fresh one must be substituted cannot be definitely stated. It is customary to continue the same starter for a week, sometimes for two weeks, but rarely for a longer period than this.

THE USE OF A STARTER

In Ordinary Cream.—In this case, the starter prepared as above described is simply added to the cream of a day's collection, in proportions which experience has found to be most useful, without previous treatment of the cream. In using the starter thus it is evidently desirable that the cream should be as fresh as possible, because the fresher the cream the smaller the number of bacteria in it, and the greater the chance that the starter will overcome them and produce its desired effect.

The use of starters in this way is open to a theoretical objection. The cream that has been brought to the creamery already contains bacteria in large numbers and, ordinarily, in considerable variety. These bacteria would themselves produce the ripening of cream, even without any starter, although it would be a slower process than when the starter was added. The effect of the starter added to the cream already filled with bacteria in great numbers will evidently not always be uniform. It may be that the bacteria already present will produce their own effect in ripening quite independent of the starter. It may be, on the other hand, that the starter added in considerable quantity will quite overcome the effect of the smaller number

of bacteria originally in the cream. If the starter added in this way is to have any effect, it must be added in sufficient quantity to overcome the deleterious action of any bacteria that may chance to be present. In practice, however, it is found that the use of starters in this way is of value and, in most cases, there is a noticeable improvement in butter made from cream thus ripened. The results, however, are not absolutely uniform, and even with the use of a large amount of starter, it will sometimes happen that the bacteria present in the cream will have more influence than those of the starter, and the butter will suffer. In this country, however, this method of using starters is becoming very widely adopted, and most of the creameries that make use of starters at all use them by the method just described. The use of starters in unpasteurized cream was first begun in this country and has been adopted more widely here than elsewhere.

In Pasteurized Cream.—Clearly, if it is possible by a simple method to destroy the bacteria already present in cream before the addition of a starter, we might expect the starter itself to have a more uniform and more pronounced effect. This can easily be accomplished by pasteurization, which, as we have already noticed, destroys a large proportion of the bacteria in milk. This method of using starters was first devised and adopted in Denmark before the method mentioned in the last paragraph was used in the United States.¹ It is the only logical method of their use. The pasteurizing of cream is simple and not very expensive, and inasmuch as it produces a medium largely free from bacteria, it is to be recommended. The use of starters in pasteurized cream has become practically universal in Denmark and some of the other countries of Northern Europe.² There are two reasons for this: 1. A higher and more

¹ Storch. *Milchztg.*, p. 304, 1890.

² Steiner. *Milchztg.*, p. 401, 1901.

Marcas. *Rev. Gen. d'Lait*, I., p. 413, 1902.

Dean. *Ann. Rep. Ind. State Dairy Ass'n*, 1903.

uniform grade of butter can be obtained in this way. 2. The prevalence of tuberculosis has brought about the enactment of a law requiring all milk that goes through the creamery to be pasteurized. For this reason Denmark butter is always made from pasteurized cream, and this makes it necessary to use an artificial starter, since pasteurized cream will not ripen of itself. The pasteurization destroys practically all of the acid bacteria, and, as we have learned, when the acid bacteria are absent the putrefying bacteria are quite sure to develop. Hence, pasteurized milk *requires* an acid starter to insure a proper ripening. Pasteurization is also said to make churning easier.

Although the method just described is the only logical method for the use of starters, it has not yet been adopted to a very great extent in the United States. A small but growing number of creameries use it, but for practical reasons it has not become popular. The pasteurization of the cream requires the purchase of new apparatus, and more work in the handling of the cream, so that it involves some additional expense. Moreover, the butter-makers have found by experience that the use of starters in cream without pasteurization in the majority of cases produces results that are quite satisfactory. Hence, whereas the use of starters, either natural or commercial starters, has become well-nigh universal in the better creameries, the pasteurization of cream to prepare it for their use has not made very much headway in this country.

In comparing the value of butter made from pasteurized cream with that made from unpasteurized cream, the former is found to be more uniform; but the butter made in this way is not, at least to the American taste, superior to butter made without pasteurization, due probably to the prevention of the growth of miscellaneous bacteria that occurs before the lactic bacteria develop. Pasteurized cream butter is somewhat milder in flavor than that made from ordinary cream, and the American market demands a flavor in its butter somewhat stronger

than that which is popular in Europe. Hence, to the American taste, up to the present time, the butter from pasteurized cream is not superior to that made from unpasteurized cream.

In pasteurizing cream for butter-making the methods already described are used. Sometimes the whole milk is run through a pasteurizer, and the cream subsequently separated from it. Sometimes the milk is first run through the separator, and the skim-milk and the cream pasteurized separately afterward. The effect of high temperature is not so marked upon cream as it is upon milk. A temperature of 180° is quite sure to produce the cooked taste in milk; but this temperature, or even a higher one, can be used upon cream without danger of developing the cooked taste in the butter, for these cooked flavors do not seem readily to affect the fat. A temperature even as high as 185° or 190° might be used without special detriment.¹ It has been thought that the use of pasteurization is deleterious to the grain of the butter, but this can be remedied by cooling the cream to a temperature of 48° before churning.

One advantage that comes from the pasteurization of cream in butter-making is that butter made from such cream *keeps* better than butter made from unpasteurized cream.² The reason for this is not difficult to understand. Pasteurized cream, subsequently inoculated with a pure culture, will contain practically nothing but lactic bacteria, and the lactic bacteria, as we have already noticed, prevent the growth of putrefactive and other organisms. The butter made from such cream will, therefore, show very few of the types of bacteria that are liable to give rise to undesirable fermentation, and for this reason the keeping property of the butter is enhanced. It is even found that the keeping of the butter is increased by the use of pasteurized water for washing the butter after the churning.

¹ Dean and Harrison. Bul. 117, Ontario Agr. Col., 1902.

² Dean and Harrison. Ont. Agri. Col. Bul. 117, 1902.

Farrington and Godfrey. Ann. Rep. Wis. Exper. Sta., 1903.

A second advantage of pasteurizing cream is that it destroys the disease germs. Butter would seem to offer an opportunity for the distribution of the germs of contagious diseases.¹ The ripening and churning do not destroy the pathogenic bacteria, and we naturally expect to find them in butter. Market butter frequently does contain the tubercle bacillus, as has been demonstrated by many examinations of market butter in Europe, and in America as well. The pasteurization of the cream is the only means that can be adopted for thoroughly protecting butter from this source of danger. It should be stated, however, that although theoretically butter may be a means of distributing any of the contagious diseases which are liable to be in milk, no data are at hand which indicate that this actually occurs. While we have many instances of epidemics due to milk, none have yet been reported that are attributed to butter. Whether this means that butter is not a source of danger, because the ripening process has injured or destroyed the disease germs, or whether it is simply lack of evidence, it is too early to state. It is significant, however, to remember that the development of lactic bacteria in large numbers has a tendency to destroy other bacteria that may be present. Inasmuch as the ripening of cream results in the enormous development of lactic bacteria, it is possible that this process actually removes from the butter some of the danger of its distributing ordinary diseases. The conclusion, however, does not apply to the tubercle bacillus.

GENERAL VALUE OF STARTERS

The almost universal adoption of starters in butter-making is in itself enough to indicate that the butter-makers have found them of value. Briefly summarized, the effect of the wide use of starters has been to raise the general quality of the butter. While perhaps the high grades of butter are not any better to-day than they were before the days of starters, there is a much

¹ Reitz. Cent. f. Bact., II., xvi., p. 719, 1906.

smaller quantity of low grade butter produced. The general result has been a raising of the average by a reduction in the amount of poor butter rather than by an improvement in the best grades of butter. In some creamery exhibits it has been found that the *starter butter* scores on the average two points higher than butter made without a starter. Very little choice is found between the commercial starters and the home starters, either of them producing a good butter and an improvement over butter ripened without a starter.

BUTTERMILK

In somewhat recent times buttermilk has come to be recommended somewhat widely as an article of diet for weak children or invalids. Buttermilk has always been used as a drink, but not until recently has it been regarded as of special dietetic value. The recommendation of buttermilk for children and invalids is a very striking comment upon the impression that bacteria are necessarily harmful. Buttermilk contains bacteria in quantities, practically as many as are in cream when ripened. The number will be from 200,000,000 to 500,000,000 per c.c., and sometimes very much higher than this. That such material, containing bacteria in these great numbers, can be recommended for children is demonstration beyond question that instead of being harmful the bacteria may be useful to human beings. The presence of acid bacteria in the human intestines seems necessary to control the normal process of digestion. If these acid germs are not present, the contents of the intestines are much more liable to undergo putrefaction, thus producing trouble. For this reason the taking of large numbers of acid organisms into the stomach seems to be very useful.¹ Buttermilk is usually almost a pure culture of lactic acid bacteria, and as such it can be used to very great advantage in cases where acid organisms are needed in the intestines. The same will be

¹ Bienstock. Ann. d. Inst. Past., xiv., p. 750, 1900.

true of sour milk, which is also frequently recommended for this purpose.

BACTERIA IN BUTTER

The relation of bacteria to butter after it is made is of less practical importance than their relation to the other milk products. Butter when first made contains large numbers of bacteria, for a considerable proportion of those in the ripened cream will get tangled with the fat. In butter, however, the bacteria do not find a medium very favorable to growth, and instead of increasing in this medium they soon begin to decrease in numbers. Their development in the first few hours is not always the same. Sometimes they will increase for a few hours after the butter is made; and sometimes they will begin to decrease in numbers at the very start. The difference is apparently due in part, if not wholly, to the amount of washing given the butter. If the butter is washed until all of the albuminous material is washed out, less food is furnished to the bacteria, the environment is unfavorable to them, and they begin to decline in numbers at once. If less thoroughly washed, the conditions are more favorable, and they may increase for a day or two. But in either case they soon begin to decline in numbers. In a single experiment, for example, the following numbers were found:

Butter, 2 hours old . . .	50,000,000 bacteria.
Butter, 1 day old . . .	26,000,000 bacteria.
Butter, 2 days old . . .	2,000,000 bacteria.
Butter, 30 days old . . .	300,000 bacteria.

In this reduction of bacteria the lactic germs are the first to disappear, and the spore-bearing bacteria are those likely to remain in the end. In old butter the number of bacteria is always small, but they never disappear, and spore-bearing organisms are sure to be found. The common dairy organism, *Oidium lactis*, is almost certain to be present in considerable numbers

in old butter, and one of the easiest methods by which a culture of this organism can be obtained is to make a common gelatin plate from a sample of old butter. From the facts just stated it is evident that bacteria will have less effect upon the butter after it is made than upon milk or cheese.

RANCIDITY OF BUTTER

It is well known that butter will not keep indefinitely. In a few days the fresh aroma of a finely ripened butter disappears, due probably to the dissipation of the volatile gases; but from this time on for many weeks the butter may remain in practically the same condition. If it is kept cool, it may be months before there is any further deterioration. But later there is an inevitable appearance of *rancidity*, and the butter slowly becomes stronger and finally unfit for use. The cause of the development of this rancidity has been difficult to determine, apparently because a variety of factors contribute to it.¹ It is, in part, probably due to chemical fermentation, produced by *enzymes* from the milk, and in part to the growth of bacteria. The rancidity is much more likely to occur if the butter is exposed to the light, and develops more readily at warm than cold temperatures. At temperatures below freezing rancidity does not occur. If butter is, therefore, kept cool and in large masses, it may be held for a long time without the appearance of any very noticeable strong flavor. In the end, however, the rancidity is practically sure to appear. To what extent bacteria are concerned in this change we do not know yet, although most investigators have concluded that they are prominently concerned in the phenomenon. There are some species of bacteria known, the *butyric acid organisms*, which have the power of producing butyric acid. *Oidium lactis*, *B. butyricus*, *B. fluorescens*, *Buttersäure bacillus* of Grassberger and other species have been found

¹ M'Kay and Larson. Bul. 71, Iowa Exper. Sta., 1903.
Rogers. Bul. 57, Bu. An. Ind., 1904.

associated with rancidity. It would be natural to assume, therefore, that they have some important relation thereto. But the change goes on after the bacteria cease to grow and may occur independently of bacteria. Rancidity may be certainly looked upon as a fermentation change, and the only method the dairyman has of controlling it is by cool temperatures, by packing the butter in large masses, and by keeping it from the light. It may be delayed by pasteurizing the cream and using pasteurized water for washing, a fact that shows its close relation to bacteria. Fortunately, it is a matter of no very great importance, because butter can be kept without difficulty for some months, and before it has spoiled it is almost always possible to market it.¹

BUTTER DEFECTS

Quite a variety of defects occur in butter, some of which are associated with bacteria, while others apparently have no connection with micro-organisms. The former are in most cases due to the growth of undesirable bacteria in the cream, producing there certain by-products, which affect the cream and butter. In some cases, apparently, the troubles are due to bacteria growing in the butter itself. The following list includes the most important of these troubles, which have been demonstrated to be associated with bacterial action.

Red Spots.—The development of red spots in butter has been studied in a few cases, and has been found to be due to a growth of micro-organisms.² In one case, a yeast, known as a pink yeast, has been found to develop somewhat abundantly in butter, producing a red color, and in other cases investigated, the cause was due to bacteria.³ The trouble is a rare one.

¹ Reinmann. *Cent. f. Bact.*, II., vi., p. 131, 1900.

Shattenfroh and Grassberger. *Arch. f. Hyg.*, xxvii., p. 54; xlii., p. 219; lx., p. 40.

Eichholz. *Hyg. Rund.*, xiii., p. 130, 1903.

Jensen. *Jahr. d. Land. d. Schweiz*, 1901.

² Weigmann. *Milch. Zent.*, I., p. 4, 1905.

³ Poda. *Milch. Zent.*, II., p. 97, 1906.

Turnipy-tasting Butter.—It has long been a belief that cows fed upon turnips will produce turnipy-tasting milk and butter. This is true to a certain extent, beyond doubt; but in some of these cases the turnipy taste has been found due not directly to the food eaten by the cattle, but to a certain kind of bacteria growing in the milk. Two or three bacteriologists have independently investigated infections of turnipy-tasting butter, and have discovered bacteria, which when inoculated into milk produce this undesirable taste. Hence, a turnipy-taste is, in some cases, of bacterial origin.¹

Cowy Butter.—The peculiar odor which is common around the barn, called "cowy odor," is well known. This odor is sometimes noticeable in milk, and it has been assumed that this is because the milk absorbs the odors from the barn or the filth that gets into the milk, which is doubtless usually correct. The same odor is sometimes detected in butter, and the interpretation has been that the odors from the barn have been transferred to the butter in this way. But it has been found that certain bacteria are capable of producing exactly this cowy odor, and there seems no doubt that in some cases this peculiar defect in butter is due not to the barn odors primarily, but to bacteria which have been transferred from the barn through the ripening cream to the butter itself.²

Putrid Butter.—This is a difficulty that is rarely seen, and has only been reported in one or two cases. Jensen found that the defect was due to a certain kind of bacteria growing in the cream and contributing to the ripening. The bacterium in question is a close ally to certain species found in the intestines (*B. putrificus*), and the putrid taste is really one of decay. This organism in question is readily killed by heat, and the pasteur-

¹ Gruber. Cent. f. Bact. II., ix., p. 684, 1902.

Jensen. Milchztg., 1892.

² Pammel. Bul. 21, Iowa Exper. Sta.

ization of the cream is a perfect remedy for the trouble. Eckles found a similar case which was due to liquefying bacteria.¹

Lardy or Tallowy Butter.—If butter is kept in the sunlight for a moderate length of time, it loses its color and flavor and becomes of a lardy and tallowy consistency. In addition to this, however, Storch has found that certain bacteria are capable of producing a similar disagreeable odor in butter.²

Oily Butter.—An organism capable of producing an oily effect on the butter, which is very common in Denmark, and produces considerable loss, has been discovered and studied by Jensen. It is an acid organism which curdles milk readily, but when growing in cream develops peculiar characteristics which give a strong, unpleasant taste. The result upon butter is quite disastrous.

Bitter Butter.—Butter with a bitter taste is a rare phenomenon, but a few cases have been discovered. Here, again, the trouble is due to the growth of bacteria in the cream. The trouble is, undoubtedly, closely associated with that of bitter milk described on a previous page.

Moldy Butter.—When butter is kept under conditions favoring their growth, molds are not uncommon upon its surface.³ These will grow especially on the outside of butter that is packed in tubs, particularly if the conditions are moist. The molds that grow are nothing more than common species, which are liable to appear on any moist organic surface, if the temperature favors their development. There is little difficulty in preventing their growth by having the tubs washed in brine. A better method still is to soak the tubs in melted paraffin, the tubs being dipped in a bath of paraffin melted at 250°. This not only prevents molding but reduces loss in the butter from evapora-

¹ Eckles. Bul. 59, Iowa Exper. Sta., 1901.

Jensen. Cent. f. Bact., ii., p. 409, 1891.

² Fischer. Hyg. Rund., v., p. 513, 1895.

Storch. 18. Rep. Danish Agri. Exper. Sta., 1890.

³ Rogers. Bul. 89, Bu. An. Ind., 1906.

tion, this reduction in loss more than compensating for the cost of the paraffin. If the butter is packed in parchment or waxed paper, soaking this also in strong brine will prevent the development of the molds. The molds do not particularly injure the butter, but detract much from its appearance.

Fishy Butter.—Butter that has been exported to Europe from Australia has frequently been reported as having a fishy taste, and a similar fishy taste has sometimes been found in American butter. In all of these instances the trouble is due to the growth of organisms. It is stated that *Oidium lactis* will sometimes produce this fishy taste in butter, but a similar fishy taste has been described by Rogers due to yeasts.

BACTERIA IN OLEOMARGARINE PRODUCTS

The oleomargarine manufacturers have found that the flavors which they wish to imitate can be produced only by the proper action of bacteria. Hence, for many years, they have adopted methods for increasing the number of bacteria, so as to obtain for their product as close an approximation to the typical butter flavor as possible. The methods by which they do this are closely related to those of cream ripening.

They first place in a vat a certain quantity of milk, cream or skim-milk; usually whole milk is used for the purpose, but frequently skim-milk and sometimes, especially for special grades, cream has been used. In this mass of milk is placed a considerable quantity of a starter, prepared essentially in the same way as before described. The whole is raised to a temperature which stimulates the growth of bacteria, and is allowed to ripen in the normal fashion. In the meantime the manufacturer prepares various fats, which he is to use to make up the bulk of his product. These fats are not always the same, different manufacturers using different materials. In this country the foundation of oleomargarine is chiefly *cotton-seed oil*, *lard* and *stearin*,

mixed in proportions to give a constituency to the final product, which is adapted according to the climate where it is to be used. If, for example, the material is to go to a warm climate a larger amount of stearin is included, while if it is to go to a colder climate, more lard. These oils are melted and mixed, and then, after the milk just mentioned above has become fully ripened, it is mixed in the oils in definite proportions, one to five being not uncommon. After thorough mixing the product is drawn off under cold brine, where it immediately hardens into a product resembling butter in most respects, except the color. The material was formerly artificially colored to resemble butter more closely. As a result of the present law the coloring matter is usually left out, and the oleo products are white. The oleomargarine itself consists of oils which have very little flavor, but the mixture is flavored by the products of bacteria growth in milk. The flavoring is, therefore, essentially identical with the flavor of butter.

As long as the manufacturers of this product were able to color their product so as to imitate butter in appearance, the product which they manufactured was very easily sold as butter. It is sometimes of such high grade that it is almost impossible to distinguish it from butter by taste or smell, and only by chemical means can it be detected. The product is perfectly healthful, and in some respects even more wholesome than butter. The manufacturers of this product are usually more careful in their processes than the ordinary farmer. The oleo contains less filth, and there is less chance of its containing disease bacteria than there is in ordinary butter. In short, so far as cleanliness and wholesomeness are concerned, a good oleo product is superior to much of the butter on the market. Now that a law practically prevents the coloring of oleomargarine it is no longer possible for it to be sold on the market as butter. It appears, therefore, as an oleo product, and in this form nothing can be said against it as a wholesome substitute for butter.

One reason that the oleomargarine products have acquired such a large market is because the manufacturers so quickly learned that the butter flavor which they wished could be obtained by the aid of bacteria growing in milk. They seized upon this idea earlier than butter-makers did, and utilized this flavor-producing power of bacteria some years before the butter-makers had awakened to the necessity of controlling their cream ripening.

CHAPTER X

BACTERIA IN CHEESE

ALTHOUGH cheese is, from a chemical standpoint, the most nutritious of all of our foods, people do not usually eat it because they realize its nutritious value. It is eaten chiefly for its flavor, since it adds a relish to many another food that may be flavorless. In this respect its value is very great, for flavors have a very important part in the economy of digestion. If we look upon cheese, therefore, both from its nutritive and its flavor value, we shall see that it is one of the most valuable of foods.

In making cheese the milk is usually first curdled with rennet, after which the curdled casein is separated from most of the water which drains from the curd as *whey*. A large part of the milk sugar is lost since it is dissolved in the whey, while most of the fat remains entangled with the casein. About two-thirds of the food material of the milk is thus retained in the cheese.

The value of the cheese is largely dependent upon its *ripening*, because it is this process which brings it into a proper condition for eating. The fresh cheese contains all the original casein of the milk, but it is rather hard, difficult of digestion and flavorless. The completed cheese is softer, easier to digest, and it contains the desired flavor.

RIPENING OF CHEESES

The ripening of cheeses is a very complex phenomenon, and one as yet only partly understood. This is due partly to the intricacy of the subject, which has presented many difficult

problems, and partly also to the fact that there are very many different kinds of cheeses, and the ripening of the different types is not by any means due to the same agency. There are no fewer than 250 varieties of cheese in France alone, and, although other countries have hardly as many, the varieties elsewhere are also numerous. These varieties may be fairly well arranged into two groups: (1) the *hard cheeses*, and (2) the *soft cheeses*, although there are some intermediate forms. The ripening of the hard cheeses is very different from that of the soft, and the ripening of the different types of the soft cheeses varies greatly one from the other. Each kind of cheese must, therefore, be studied as a special problem, and while the ripening of some kinds of cheeses has been thoroughly studied and is quite well understood, that of others remains wholly for the future to explain. At the present time, therefore, we can give only some general statements regarding cheese ripening and its relation to micro-organisms.

To understand the phenomenon in question we notice, first, that the changes occurring in the ripening can be divided into three classes: (1) physical changes affecting the general appearance of the curd; (2) chemical changes affecting its composition; (3) chemical changes which develop the cheese flavors.

Chemical and Physical Changes.—These may be considered together, because they are doubtless closely connected. A very noticeable physical change takes place in the texture of the curd. When first made this is hard, rather dry, and somewhat tough and elastic; but as the ripening occurs its texture changes, its elasticity disappears, and it turns eventually into a softer constituency, which differs very much in the different types of cheeses. The softness of the resulting cheeses is dependent largely upon the amount of moisture it contains and the temperature. The larger the amount of moisture left in the cheeses, other things being equal, the softer it will become; and the higher the temperature at which the cheese is kept, the softer

it tends to become. Dry cheeses are always hard, and even the soft cheeses may be hard after they become dry.

The chemical changes which occur are very profound. The first to occur is, in most cases, the development of lactic acid, and in nearly all, if not all, types of cheeses this souring is necessary for the subsequent steps. After the acidity develops the changes go on very much more slowly; but they usually consist of a gradual reduction in the amount of acidity until finally the cheese may become slightly alkaline. At the same time the casein is undergoing a series of changes by which it is gradually reduced from the condition of a raw proteid (casein) to the condition of peptone or proteoses, which are similar products. Proteids are, as a rule, insoluble in water, but after they have been converted into peptones or proteoses, the products are soluble. This change is essentially similar to that occurring in the *digesting* of the proteids in the stomachs of animals, and the changes in the ripening of cheese are very similar to those of digestion. For this reason a ripened cheese is to be looked upon as an already digested food. With several of the soft cheeses, like the Brie and Camembert, the digestion is very complete, and the ripened cheese is an almost wholly predigested product. Such cheeses are very easily handled by the digestive organs and form a very easy assimilable food. The extent to which this breaking down of the casein into soluble products occurs varies very much in the different types of cheeses. In the hard cheeses it is less complete than in the softer ones; and in the latter class the change may be so great as to reduce the casein largely into soluble products. The change also varies with the age of the cheese, for it is a phenomenon that progresses week by week as the cheese ripens and does not become complete until the whole process is finished. This general process is commonly called *digestion*.¹

¹ Van Slyke and Hart. Ann. Rep. N. Y. Exper. Sta., 1903.

Van Slyke and Hart. Bul. 261, N. Y. Exper. Sta., 1905.

Harding. Bul. 237, N. Y. Exper. Sta., 1905.

Flavors.—The production of flavors is of no less importance than the chemical digestion of the cheese. At the present time, however, there is a very profound ignorance concerning the real source and cause of cheese flavors. They are without doubt the products of decomposition. They appear in the cheese only toward the end of the ripening process, and are regarded generally as due to the end products of decomposition. The proteids and proteoses, that result from the digestion of the caseins, do not themselves have any of these peculiar cheese flavors; but toward the end of the ripening some of the material seems to be still further broken down into the simpler end products which show the flavors characteristic of cheese. The nature and cause of these flavoring substances we do not definitely know, but further reference to the matter will be made later.

BACTERIA IN CHEESE RIPENING

In studying the relationship of micro-organisms to cheese ripening, we can best understand the subject if we consider separately the chief types of cheese. There is no question but that the ripening processes of hard and of soft cheeses, although phenomena somewhat closely related, are very different from each other, and are produced by very different agents. We shall understand the subject better if they are considered separately.

Sour Milk Cheese, Dutch Cheese, etc.—There is one type of milk product, commonly called cheese, that is not in any proper sense a true cheese. Under this head are included cheeses that consist of nothing except the curd of sour milk. In making them the milk is allowed to sour under the action of the lactic acid bacteria until it is well curdled.¹ Sometimes, however, a little rennet is added to the milk in order that the curdling may occur more quickly than it would under the action of the lactic bacteria alone. After being thus curdled, the curd is separated

¹ Hall, Van Slyke and Hart. Bul. 245, N. Y. Exper. Sta., 1904.

from the whey by draining and finally by squeezing, by various processes, until it is reduced into a fairly hard pulp. It may then be run through mills which grind it up into a very fine, smooth mass. This product may be molded into cakes or short cylinders and wrapped in tinfoil or paper, to be sold at once and *aten fresh*. This type of cheese has long been known in our households as *Dutch cheese*, *cottage cheese*, *Smearcase*, etc. Such cheeses seem to be easily digested. They are the result of the action of bacteria upon the milk with no other agency seemingly concerned, and are primarily due to the growth of the lactic acid species. In recent years, our dairymen have developed a large market for two very similar cheeses, which they, however, sell under the name of *Neufchatel*, and *Cream cheese*. These two cheeses, so abundant in our markets, are scarcely any different from the old cottage cheese of our homes, except that rennet is used to hasten the curdling, and the curd is afterwards ground between rollers in a mill, which renders it smooth. American *Neufchatel* cheese is, therefore, nothing more than a sour curd, not ripened, and having no real cheese flavors; a very different product from a European *Neufchatel* cheese. This product should not really be classed under the head of cheeses, but as a sour milk curd. Sometimes such sour milk curds are ripened into a real cheese (*Harz cheese*), but this is not done in America.¹ The ripening that occurs is similar to that of some of the soft cheeses described below.

THE RIPENING OF HARD CHEESES

Hard cheeses are much more used in the United States than any of the soft varieties. The most common kinds are the *American* or *Cheddar* cheese, the *Edam* cheese, the *Pineapple* cheese, and the *Swiss* cheese. In all of these cheeses the essential feature of the manufacture is that after the milk has

¹ Eckles and Rahn. Cent. f. Bact., II., xiv., p. 676, 1905.

Eckles and Rahn. Cent. f. Bact., II., xv., p. 726, 1906.

been curdled by the use of rennet, the whey is extracted *as completely as possible*, first by cutting the curd and draining, and then by pressing under a high pressure until all of the whey which can be squeezed out is removed. This makes a hard, tough mass, which is put aside for ripening. The details of manufacture differ, of course, in different types of cheeses. In the Swiss cheese the milk is sweet when it is curdled, while in the American or Cheddar cheese the milk is allowed to become sour. Each of the other types of hard cheeses has its own special method of manufacture, with slight differences in the product.

The ripening of these cheeses involves both digestive and chemical changes, and the production of flavors. To bring about these changes there are, at least, two different agents, either or both of which may play a part in the process. The cheeses contain both *enzymes* and *bacteria*.

Enzymes.—In all cheeses there is present a certain quantity of enzymes, capable of digesting casein, derived from, at least, two sources: 1. The rennet used in cheese curdling is derived from the stomachs of sucking mammals, and because of its method of preparation rennet will always contain considerable quantities of *pepsin*, the juice that carries on the digestive functions in the mammal's stomach. It is impracticable to prepare rennet from a stomach without its containing some pepsin. 2. It has also been shown by Babcock and Russell that milk itself,¹ when secreted, contains a ferment, which they have called *galactase*, and which has also the power of digesting casein. This is either secreted by the cow or by bacteria growing in the milk ducts of the udder. Both pepsin and galactase are capable of converting casein into peptones or proteoses, and during the ripening period they are both acting slowly but constantly. By some it is believed that they are, to a large extent, responsible

¹ Babcock and Russell. Ann. Rep. Wis. Exper. Sta., 1900.

for the changes of casein, which constitute the ripening of the cheese.

Bacteria.—The cheeses also contain bacteria in great numbers, and usually in some considerable variety. Bacteria are present, of course, in the original milk, and when the milk is placed in the vat for curdling, their number will be considerable, differing very slightly in the different kinds of cheeses. When the cheese is made of sour milk (Cheddar Cheeses), their number will be very high, for the reasons already indicated, whereas, if cheese is made of sweet milk (Swiss Cheese), the number will naturally be much less. Bacteria, too, are added to the milk with the rennet which always contains vast numbers, some of which are useful, some detrimental and some indifferent.¹ It has even been suggested that the useful bacteria (acid bacteria) be artificially added to rennet to hold the others in check. The bacteria which are in the cheese at the beginning grow rapidly. For some days after the cheese is made they increase rapidly in numbers, reaching a maximum usually in about five days, when they may be as many as 650,000,000 per gram.² After this they decrease. The decrease in cold-cured cheeses is less rapid than in those cured at a higher temperature. The bacteria found are chiefly lactic acid bacteria, two common types being found, the others that may be present at the outset soon disappearing.³ During the ripening they lose their acid-producing power to a considerable extent, this fact perhaps favoring the development of taints.

Chemical Changes.—There are thus, at least, two different factors that may contribute toward the chemical changes of ripening cheeses—the growth of bacteria and the action of the chemical ferments. Which of these two factors is the prominent one in cheese ripening is a much discussed question.

¹ Thöni. *Land. Jahr. d. Schweiz.*, 1906.

² Harrison and Connell. *Rev. Gen. d'Lait*, III., 1903.

Harrison. *Rev. Gen. d'Lait*, V., p. 409, 1906.

³ Wenzirl. *Cent. f. Bact.*, II., vi., p. 785, 1900.

When cheese ripening was first studied by Duclaux, it was believed by him that the ripening was primarily the action of bacteria. We have already seen that certain kinds of bacteria have the power of changing casein to peptone—the liquefying type (see page 40)—and Duclaux, finding such organisms more or less abundant in cheeses, reached the conclusion that the change from casein to peptone in the cheese ripening was due to the growth of these peptonizing bacteria. This he argued strongly and held for many years, supporting his position by the claim that when treated by heat or antiseptics, so as to destroy bacteria, the bacteria would not ripen. Later the fact was more and more clearly seen that the liquefying bacteria are not common in cheeses, especially in the better grades. If present at the beginning they rapidly decrease in numbers and come to be very few, if not absent entirely, a fact which forced the conclusion that they cannot contribute materially to the ripening of cheeses. More recently, Gorini has advanced the view that certain "acid liquefiers"—i.e., peptonizing bacteria that at the same time produce acid—are intimately connected with the ripening.¹ But there does not yet appear to be much evidence for this.

These facts led to a suggestion by Freudenreich that the ripening is due really to the lactic acid bacteria.² Lactic acid bacteria, as we have already seen, do not liquefy gelatin and do not ordinarily have any power of changing casein to peptone. As they grow in the milk, they produce lactic acid which curdles the milk, after which they apparently cease to act upon it at all. Hence, it would not seem that they could digest cheese. Freudenreich,³ however, showed that if the acid which they produce

¹ Gorini. Cent. f. Bact., II., viii., p. 137, 1902.

Gorini. Rev. Gen. d'Lait, iii., pp. 289, 405, 1904.

² Freudenreich. Tirage special d. l'Ann. agricole de la Suisse, 1905.

³ Freudenreich. Rev. Gen. d'Lait, I., p. 112, 1901.

Freudenreich. Land. Jahr. d. Schweiz, 1897, 1899.

Freudenreich and Thöni. Cent. f. Bact., II., xiv., p. 34, 1905.

is neutralized by the presence of some alkaline, like carbonate of soda, the bacteria continue to grow, and eventually produce the peptonization of the casein. Moreover, he was able to show that the grade of the cheeses is very closely dependent upon the growth of lactic bacteria. He found that certain types of lactic bacteria, inoculated into the cheese, resulted in a fine grade of cheese, while others resulted in a poorer product. He found, also, that cheeses from which lactic acid bacteria were excluded by aseptic milking would not ripen normally, while they would do so if the acid germs were present.¹ All of these facts together led him to the conclusion that it is this peptonizing power of the lactic acid bacteria, under certain conditions, which is responsible for the chemical changes that take place in the ripening cheese.² This conclusion, however, has not been very generally accepted; for while others have confirmed his conclusion that the lactic acid bacteria under these conditions do produce a certain amount of peptonization of the casein, the action is extremely slow and not very complete, and it has not seemed to most chemists that the phenomenon in question is sufficiently explained by this slow action of the lactic acid bacteria. The bacterial explanation of the ripening has been thrown into uncertainty by Babcock and Russell, who showed that cheeses, if kept in a vapor of chloroform, which will prevent bacteria growth entirely without checking the action of the enzymes, will undergo the chemical changes of ripening, although they will not acquire the desired flavor.

As a result, a third theory has been advanced that the chemical changes of digestion are due to the action of the enzymes.³ These enzymes are always present, some in the original milk and some added with the rennet, and it is only natural to suppose that during the long period of ripening they will carry on

¹ Freudenberg. *Land. Jahr. d. Schweiz*, 1902.

² Jensen. *Milch. Zent.*, II., p. 393, 1906.

³ Babcock, Russell and Vivian. *17. Ann. Rep. Wis. Exper. Sta.*, 1900.
Jensen. *Landw. Jahr. d. Schweiz*, 1900.

the peptonizing of the casein, which is their distinctive characteristic.¹ The conclusion regarded as most probable to-day, therefore, is that the chemical changes, whereby the caseins are broken down to peptones and proteoses, are due to enzymes' action rather than to bacteria, and the enzymes that produce the action are partly present in the original milk (galactase), and partly added to it with the rennet, which is used to curdle the milk (pepsin).²

Flavors.—The source of the cheese flavor is even more uncertain than that of the chemical action. The peptones and proteoses, which are obtained by the enzyme digestion of the caseins, do not possess any noticeable flavor, and certainly do not show any trace of cheese flavor. The digestion of the casein, which may have been produced by the enzymes present, will not result in the flavoring of the cheeses. Moreover, the cheeses ripened in chloroform vapor, which allows the enzymes to act, but prevents bacteria from growing, though they ripen, do not develop flavors.³ These flavors must be due to some other cause than enzyme action. That they are the end products of chemical decomposition seems to be extremely probable. In many cases, they are associated with ammonia; and ammonia, as is well known, is one of the final products of proteid destruction. It is generally believed that these end products are the result of the action of bacteria or some other micro-organisms. The only known agency that commonly produces the complete destruction of proteids is bacteria, and while the matter has never been put to any satisfactory test, the most probable explanation seems to be that these cheese flavors are the result of the final bacterial decomposition.

Against this view, however, has been urged the fact that in

¹ Van Slyke, Harding and Hart. Bul. 203, N. Y. Exper. Sta., 1901.

² Marcus and Huyge. Rev. Gen. d'Lait, vi., p. 27, 1906.

³ Van Slyke and Hart. Bul. 231, N. Y. Exper. Sta., 1903.

Harding. Bul. 237, N. Y. Exper. Sta., 1903.

Van Slyke, Harding and Hart. 20. Ann. Rep. Wis. Exper. Sta., 1902.

the well-ripened cheeses hardly any bacteria, except lactic acid organisms, are present, and this class of bacteria does not, so far as is known, have any power of producing cheese flavors. Some bacteria, if they grow in proper abundance in milk, will in time develop well known cheese flavors; but these organisms have not been found in old, strongly flavored cheeses. Whether they have anything to do with the production of cheese flavors is therefore uncertain. It has been suggested by Rogers¹ that the flavor of cheeses is due to the bacteria which grow in them during the first few days. Liquefying bacteria are found during this early period, and before the miscellaneous bacteria disappear, as they do later, some of these liquefiers may secrete from their bodies substances, possibly enzymes, that continue their action in the cheese slowly, but for a long time. Although the bacteria that produce them soon die, the chemical ferments which they have produced continue their activity until they finally produce the new products that give the flavor. On the other hand, Harrison and Connell² think the lactic bacteria most closely associated with flavor. All of this, however, is at present largely theoretical. We know that the digestion of the casein, whether produced by pepsin or by galactose, does not produce the cheese flavors. We know that certain kinds of bacteria are capable of producing these flavors, and while the matter has not yet been settled, the probability seems to be that the flavoring of the cheese must be attributed to bacterial action.

Other Functions of Bacteria in Cheese-ripening.—Whether or not bacteria are the source of the flavor of hard cheeses, there is no doubt that they do play a very important part in cheese-making in several distinct directions. As has already been indicated, the first step in the ripening of the cheese is the souring of the curd, and this is produced by lactic acid bacteria. It seems to be quite necessary that this should take place in

¹ Rogers. Bul. 62, Bu. An. Ind., 1905.

² Harrison and Connell. Cent. f. Bact., II., xi., p. 637, 1904.

order that the proper chemical changes be obtained. In the manufacture of the Cheddar cheese the development of a proper acidity in the curd is necessary to produce the proper texture in the cheese, and, also, to bring about the proper ripening. If the milk sugar is washed out of the cheese so that lactic acid cannot be formed the ripening does not progress normally; and very badly ripened, badly flavored cheeses are the result.¹ This is due to the fact that in such cheese the acid bacteria do not grow, while the liquefiers develop abundantly. A study of the chemistry of the ripening of cheeses has shown that the conversion of the casein into the desired digested products occurs only when lactic acid is present to combine with them in forming certain new products. Thus the development of lactic acid in the cheese is a necessary first step in the ripening, and inasmuch as this lactic acid is dependent upon lactic bacteria, the ripening is closely associated with these organisms. Weigmann believes further that the lactic bacteria prepare the way for other species that grow later and furnish the flavors; but this is not yet proved.

In the ripening of Swiss cheeses the relation of bacteria is somewhat different. These cheeses, as already noticed, are made from sweet milk rather than sour. The Swiss cheeses when properly ripened contain large holes, a distinctive characteristic of this peculiar type. These holes seem to be due to the carbon dioxide gas produced by certain types of bacteria, but it is not the common lactic acid bacteria that produce them, for these do not give rise to gas. Nor does it seem to be the common gas-producing lactic bacteria referred to on page 34; for these, as noticed later, are apt to ruin cheeses. The normal holes of the Swiss cheeses are due to a different species of bacterium, which has recently been described.² But nevertheless the Swiss school of dairymen has learned that the ripening

¹ Babcock and Russell. *Cent. f. Bact.*, II., ix., p. 757, 1902.

² Freudenreich and Jensen. *Cent. f. Bact.*, II., xvii., p. 529, 1906.

can be very much better controlled if the curd contains a culture of proper lactic acid bacteria, although it is not yet known exactly what action they have in the ripening.

As a result of these facts, cheese makers have in recent years learned that the use of lactic acid starters in their milk is decidedly advantageous. This has been independently worked out and adopted in Europe and in America, and in the last few years the practice of using pure lactic starters in milk which is to be made into cheese has been rapidly growing. It has been found that this practice enables the cheese maker to control much more accurately his ripening cheese and to reduce the number of failures. The reason why the inoculation of a lactic starter tends to reduce the failures in cheese-making can be easily understood from the facts already considered. The lactic acid bacteria have the power of checking the growth of other germs, and even of destroying them entirely. When, therefore, the milk has a large quantity of lactic bacteria developing rapidly in the curd, the other bacteria, which might, under different circumstances, produce unpleasant flavors and tastes, thus injuring the cheese, are prevented from increasing. The lactic bacteria protect milk from putrefactive and other changes which would result from the growth of miscellaneous bacteria; but in the handling of milk this is a matter of little importance, for the milk is used so quickly. In the handling of cheeses, however, this protecting action of the lactic bacteria becomes very much more important and is indeed the secret of good cheese. The cheese curd lies for weeks, or even months, in a moist condition, and there is opportunity during all this time for the growth of bacteria. If a proper lactic organism is present at the outset, the cheese will be protected from the various putrefactive types that would otherwise be likely to injure it. Their absence in sufficient quantity is responsible for many of the defects to be noticed later.¹

¹ Peter. *Cent. f. Bact.*, xiv., p. 321, 1905.

For these reasons, then, the cheese industry is learning the prime importance of a strong lactic fermentation in milk to be made into cheese, and in order to bring this about it is rapidly adopting the method of using starters. The starters used in cheeses are essentially identical with those used in butter-making, and they are used in much the same way. *Home starters* are frequently made and inoculated into the milk, and the use of *commercial starters* is also rapidly growing. It has been suggested that acid bacteria be inoculated into the rennet to prevent the growth there of undesirable bacteria. It is interesting to find that types of lactic bacteria which are useful in butter-making are not always satisfactory in cheese-making. In some experiments, for example, it has been found that bacteria that give a fine flavor and aroma to butter, produce a bitter taste and a ruined product when used in cheese-making. This simply indicates that the types of lactic bacteria are different, at least so far as concerns their final action on the casein. The use of starters in the cheese industry seems to be firmly established at the present time, and is practically sure to extend, for it is one of the methods of safe-guarding cheese against undesirable fermentations; and it is almost sure to become recognized as even more necessary than in butter-making.

BACTERIA IN EDAM CHEESE

It is an interesting fact to learn that, some years before the relation of bacteria to cheese-making was studied, the Holland cheese makers had practically learned the value of bacteria cultures in making Edam cheeses.¹ For many years they have used a starter known as *slimy whey*; which was added to the milk that was to be made into cheese. This material contains a large quantity of a species of a coccus, *Strep. Hollandicus*, that makes the milk slimy, and when used in cheese-making regulates the ripening so that the cheeses mature more rapidly and more

¹ Bockhaut and Ott de Vries. Rev. Gen. d'Lait, v., p. 1, 1905.

uniformly than when made without such a starter. Not all Edam cheeses are made by the use of such a slimy whey, but its use has become quite prevalent. The cheeses made from it are no better than the best cheese made without it, and indeed some think they are not quite so good; but they are more uniform, there is less chance for failure, and they ripen in four weeks instead of six. It has been recently shown that the use of a pure culture lactic starter in Edam cheese works just as well as the slimy whey.

PASTEURIZATION IN CHEESE-MAKING

In the manufacture of hard cheeses the use of pasteurization as a method of preparing the milk for the reception of a starter has not been successful. Pasteurization has an effect upon milk, such that it does not curdle by rennet in exactly the same way that it would otherwise do. This matter is one of comparatively little importance, for the curdling property can be restored by the addition of a calcium chloride to the milk. But experience has shown that, for some reason as yet unknown, cheeses made from pasteurized milk will not ripen normally. Whether this is due to the fact that pasteurization destroys certain of the enzymes necessary for ripening, or to the fact that it kills some of the necessary micro-organisms, or to both facts combined, is not yet ascertained. Pasteurization, however, has hitherto been found impracticable for the hard cheese industry. What may develop in this line in the future cannot be stated.¹

Temperature of Ripening.—The ripening of hard cheeses is a slow process, requiring weeks and sometimes months before completion. The higher the temperature, the more rapid the ripening, and the less the uniformity² of the cheeses. The

¹ Milchztg., p. 582, 1904.

Henzold. Milchztg., p. 262, 1901.

Tiemann. Milchztg., p. 386, 1901; p. 212, 1902.

Klein and Kirsten. Milchztg., p. 6, 1901.

² Farrington and others. Ann. Rep. Wis. Exper. Sta., 1903.

Babcock, Russell, Vivian and Baer. Ann. Rep. Wis. Exper. Sta., 1901.

See also Bul. 49, Bu. An. Ind., 1903, and 20. Ann. Rep. Bu. An. Ind., 1903.

common practice of leaving the cheese in a fairly cool room to ripen at normal temperatures has produced a fairly rapid ripening, giving a more or less strong cheese of the type that has been common on the market. In the last few years, however, experiments have been undertaken to determine the effect of ripening at lower temperatures. The results have been highly satisfactory, for it is found that if the cheeses are placed in ripening rooms at temperatures below 56°, though ripening goes on much more slowly, it is more uniform, more satisfactory, and there is a noticeable improvement in the product. The use of low temperatures for ripening is quite new, but has extended rapidly, and at the present time a large amount of cheese is thus ripened.

DEFECTS IN HARD CHEESES

Most of the defects which injure a cheese, sometimes completely destroying it, are due to the growth within it of undesirable organisms. There is quite a variety of these, some of which are rare, some of which are fairly common, and some of which cause immense losses to cheese makers the world over. The most important we will briefly consider.

Gassy or Swelled Cheese.—The phenomenon spoken of as gassy or swelled cheese is perhaps the most common of cheese faults. It is due to the development inside of the curd of a considerable quantity of gas, which fills it full of holes and causes it to swell and lose its shape. Sometimes the holes are extremely numerous and small, and sometimes they are fewer but of larger size; and, in any case, they are undesirable. The abundance of the gas produced varies greatly. Even good cheeses are apt to show some gas holes, but the amount is so small it does no especial injury to the cheese. Sometimes the gas is so abundant as to cause the cheese to burst, in which case it is, of course, completely ruined. Between these extremes are all kinds of intermediate grades. In some

cases, the development of the gas is accompanied by an unusual fermentation and an unpleasant taste and smell. The appearance of much gas always injures and sometimes totally ruins the cheese. The cause of the trouble is the development of gas-producing bacteria. Several different species are known, which have this power of developing gas in great quantities in



FIG. 61—PIECES OF CURDLED MILK

One curdled by the common *Bact. lactis acidi*, showing no gas; the other by *Bact. aerogenes*, filled with gas bubbles that would spoil a cheese

the ripening cheese. Most of them, perhaps all, belong to the type which have been referred to in Chapter II as *Bact. aerogenes* type. (Fig. 61.) As pointed out in that chapter, the different varieties of this type vary much in the amount of gas they produce; sometimes the quantity is very slight, sometimes it is extraordinarily large, and it is easy to understand how different strains and different quantities of bacteria will produce grades of gasiness in cheeses. It is to be remembered that the gas which produces the swelled cheese is a different thing from the gas which produces the normal holes in the Swiss

cheese. Swiss cheeses also suffer from gas production, but the phenomenon is totally different from the one which produces the ordinary holes so familiar in that type, and is caused by different species of bacteria.

Remedies for gassy cheese are chiefly in the way of prevention, not of cure. Indeed, it may be stated concerning all the defects of cheeses that, after they have once made their appearance, there is practically no remedy. They are slow in developing, and usually are not seen until the cheese is so far ripened that the injury is beyond repair. The cheese makers' defence is in guarding against them; and for this purpose various methods are within his reach. One of the most satisfactory is the use of a large, vigorous starter of common lactic acid bacteria. The protecting power of this type of bacteria will prevent the growth of many others, and if the non-gas-producing acid organisms are present in the milk in sufficient quantity, the gassy organisms will be held in check. A vigorous culture of slimy whey is said to be efficient in preventing swelling of Edam cheeses, and it will do the same in other types of cheese.¹ A second factor in preventing gas formation is the use of low temperatures. As pointed out on a previous page, the gas-producing organisms develop readily at high temperatures, 80° to 100°. At lower temperatures, on the other hand, they do not grow very readily, while the *Bact. lactis acidi* type does grow vigorously. If, therefore, the curd is kept at low temperatures, the development of the gassy curd and the gassy cheese is much lessened.² Cheeses that will become gassy at 75° will not do so at 60°. It is possible by a cool temperature so to check the development of gas that a fair cheese may be produced from milk, which contains enough of gas bacteria to ruin it. A third partial preventive is the use of more salt in the cheese, for this

¹ Bockhaut and Vries. Cent. f. Bact., II., xii., p. 89, 1904.

² Spallanzani. Rev. Gen. d'Lait. IV., p. 477, 1905.

in a measure prevents the development of the gas organisms. A fourth preventive is the use of the ferment or curd test of the samples of milk, as shown on page 258. By the use of this ferment test the cheese maker can discover whether the milk from any of his patrons contains too large a number of gas-producing organisms. If so, he can discard the suspicious milk, and thus avoid danger of excessive gas production in his general product.¹

Bitter Cheese.—Large losses have been produced in cheeses by the development of a bitter taste, a trouble probably always due to the growth of micro-organisms. In one extended infection of this sort, the bitterness was traced by Harrison to yeasts (*Torula amari*) found in the vats, in the cans, and other utensils which held the milk to be made into cheese.² (Fig. 26.) A thorough cleaning and sterilization may be efficient in removing the difficulty. In other cases, a similar trouble has been due to the action of bacteria from sources not yet understood, developing vigorously and producing strong, bitter-tasting products.³ One of the common sources of such trouble is the liquefying bacteria. These, as we have seen, are always likely to be in the milk, but their growth in the cheese is held in check by the lactic acid bacteria. Sometimes the acid organisms are not vigorous enough to restrain them, and they develop too abundantly. As we have earlier noticed, they may produce bitter products, and these affect the cheese. Large losses are sometimes due to such a cause, the whole output of a factory being affected in this way for a long time. Remedies against these troubles can be found only on the same

¹ Freudenberg. *Land. Jahr. d. Schweiz*, 1890.

Weigmann. *Land. Woch. f. Schlesw.-Holst.*, 1890.

Thöni. *Land. Jahr. d. Schweiz*, 1906.

Hastings. *22d Ann. Rep. Wis. Exper. Sta.*, 1905.

Russell and Hastings. *Bul. 128, Wis. Exper. Sta.*, 1905.

Weigmann. *Milch. Zent.*, II., p. 441, 1906.

² Harrison. *Rev. Gen. d'Lait*, I., 1902.

³ Freudenberg. *Land. Jahr. d. Schweiz*, 1894.

Weigmann. *Milch. Zent.*, II., p. 441, 1906.

lines already pointed out,¹ cleaning of the milk vessels, more care in the production of the milk, and the use of larger quantities or more vigorous starters of lactic acid organisms, to check the growth of other bacteria.

Putrid Cheese.—Sometimes milk becomes acted upon by putrefying bacteria; soft spots appear upon the surface, which may become larger and eat their way into the cheese, and produce a more or less slimy appearance upon the cheese, even to the center. The extent of the trouble will depend upon the abundance of the putrefaction. The trouble is undoubtedly due to the growth of putrefying bacteria, but not much is known about the matter at present.

Fruity or Sweet Cheese.—This is a phenomenon which occurs sometimes over widely extended districts, and detracts from the character of the cheese without always ruining it. It is characterized by a peculiar sweet taste, which, although not unpleasant, spoils the flavor of the cheese and thus injures the sale of the product. This trouble has been studied by Harding and found to be due to a yeast, which gets into the milk.¹

Rusty Spot of Cheese.—This is a defect occurring in the cheese during the ripening, characterized by rusty, red spots on the outside and, indeed, not infrequently throughout the whole cheese. The cheese loses its value, and may in the end become quite ruined, if the trouble develops sufficiently. The cause is a bacterium, *B. rudensis*, which has been studied by Harding and Connell.² In all these cases the only remedy is the preventive one of a greater guard placed over the milk supply, a more careful cleaning and steaming of the milk vessels, until the organisms producing the troubles are eliminated.

¹ Harding, Rogers and Smith. Bul. 183, N. Y. Exper. Sta., 1900.

² Harding, Rogers and Smith. Bul. 183, N. Y. Exper. Sta., 1900.

Harding and Smith. Bul. 225, N. Y. Exper. Sta., 1903.

The difficulty in dealing with all cheese defects is that one cannot be sure, until weeks after he has applied his remedy, whether it is sufficient to eradicate the trouble. The defects usually appear weeks after the cheese is made; if, therefore, the trouble should appear, and a dairyman should institute a most careful renovation and cleaning of his vats and cans, it will be weeks before he can be sure that he has reached and removed the difficulty. This makes the handling of the cheese problem an extremely difficult one, and it emphasizes strongly the lesson that only by constant watching can one be successful in producing uniformly a good product. More than any other dairy product is cheese dependent upon the presence and growth of the proper micro-organisms.

SOFT CHEESES

The essential difference between a hard cheese and a soft cheese is the quantity of water left in the cheese after the making, the soft cheeses having a very much higher percentage of moisture. To bring about this condition the method of manufacture is designed to *retain* the whey in the curd. After the milk is curdled the curd is sometimes dipped out directly into forms provided with holes in their sides, through which the whey drains naturally without the application of any pressure. (Fig. 62.) In other cases, the curd is cut in the vat, but the curd and whey together are dipped into forms for draining. In all cases, the whey simply drains naturally without pressure. As a result, there is produced a cheese which is softer in texture and which contains a very much higher quantity of water than is allowed to remain in the hard cheeses.

This large amount of water produces a ripening of a totally different character from that which occurs in the hard cheeses. The process of ripening is different, the agents that bring it about are different, and the final result is very dif-

ferent from that in the hard cheese; moreover, the ripening is liable to greater variations in the soft cheeses than in the hard cheeses and is more difficult to control. The difficulty of controlling the ripening can be readily understood when one appreciates the effect of the large amount of moisture present. Bacteria, molds and yeasts all find a suitable medium for growth in the wet curd of the soft green cheese, and unless

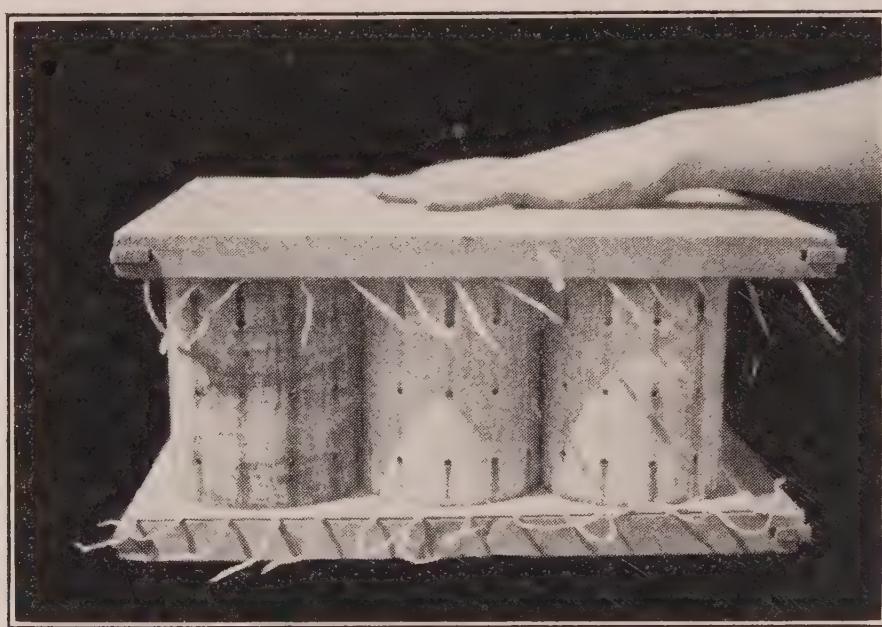


FIG. 62—FORMS USED IN MAKING AND DRAINING CAMEMBERT CHEESES

the progress of the ripening is exactly right, the cheese maker may expect the development of kinds of micro-organisms that are unfavorable to his product and that will spoil his cheeses. The experience of soft cheese makers bears out this theoretical conclusion, for the variety of soft cheeses is very great, and a large number of cheeses are ruined by improper ripening, due to the growth of undesirable organisms. Soft cheeses are much less uniform in character than hard cheeses. They differ very greatly in texture and in flavor, and are subject to a large number of defects that injure or ruin them. They are, in

short, more difficult to make with success than the hard cheeses, largely, if not wholly, because the water they contain offers such a favorable medium for the growth of bacteria and other micro-organisms. Partly for this reason, also, they are the higher priced.

Two or three types of soft cheeses have been carefully studied, and their ripening is fairly well understood. We may, therefore, give in some little detail the facts known concerning the ripening of three of these types of soft cheeses, the three selected being those that are most popular in our markets, and those which bring the highest prices: the Camembert, the Roquefort and the Limburger. These types have been subjected to a more thorough investigation, and more is known concerning their method of ripening than of any other kinds of cheese. Roquefort cheese is not strictly a soft cheese, since the curd is pressed, but it is softer than the typical hard cheeses, and moreover its ripening is more like that of the soft cheeses than the hard. Hence, we class it here. Of these three types, one is ripened by molds primarily, one by molds and bacteria together, and the third by bacteria without molds.

THE CAMEMBERT TYPE

This includes the Camembert and the Brie cheeses, which are essentially alike, differing only in size and shape, and sometimes in their amount of fat, but made and ripened in the same way.¹ The French Coulommier cheese also belongs to the group, and the *ripened* Neufchatel. The latter is not found in this country. The ripening process is essentially the same in all cases; all have a moldy rind, and the resulting cheese is very much alike. These cheeses were first manufactured in France, where they have been made for a century; but from that country they have been distributing themselves

¹ Epstein. Arch. f. Hyg., XL., p. 1, 1902; XLV., p. 354, 1902.
Thom. 22d Ann. Rep. Bu. An. Ind., 1905.

over the neighboring countries in Europe. An attempt has also been made to manufacture them in America, but hitherto with only partial success.¹ While it has been possible to manufacture cheeses with the same qualities as Camembert, it has hitherto been impossible to do so with sufficient uniformity to

make the industry a perfect success. The reason for the lack of uniformity is quite easy to understand when one has a knowledge of the real nature of the ripening.

The ripening of the Camembert cheese, the best illustration of this type, has been so thoroughly studied that it is fairly well understood. The first phenomenon that occurs is the souring of the curd, which is brought about by a *Bact. lactis acidi* type of organism. These grow in the milk previous to the addition of the rennet, although the milk is not allowed to become very sour before curdling. But they continue to grow

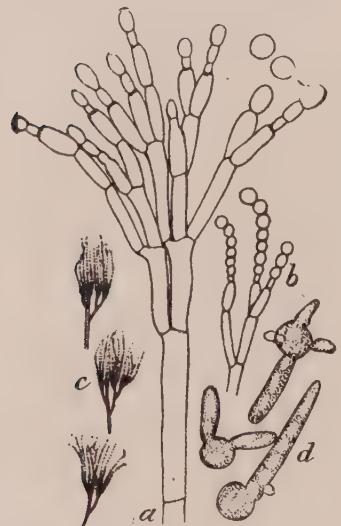


FIG. 63—*PENICILLIUM CAMEMBERTI*

Showing method of forming spores (Thom.)

during the curdling and for a day or two after the cheese is made. If by any chance the lactic bacteria fail to develop vigorously, a ruined cheese is sure to result.

The second step in the ripening is the appearance on the surface of the cheese of a species of mold, which has been named *Penicillium camemberti*.² (Fig. 63.) This mold appears in from two to four days and is, at first, of a pure white color; later, when it begins to produce spores, it becomes a steel gray, but never a deep blue like the common mold. (Fig. 64.) It is a species of mold that apparently does not occur in

¹ Conn, Thom and others. Ann. Rep. Storrs Exper. Sta., 1905.

Thom. Ann. Rep. Storrs Exper. Sta., 1905.

² Thom. Ann. Rep. Storrs Exper. Sta., 1905.

America, but it is very common in Europe in the sections where Camembert cheese is made. Its absence from America is the chief reason why America has been unable to make Camembert cheese; and where successful cheeses have been produced, it has been by importing and inoculating this species of mold



FIG. 64—FOUR COLONIES OF A COMMON MOLD ON SOFT CHEESE
Penicillium camemberti

into the American cheese factories. This white mold grows on the surface of the cheese, but does not penetrate below the surface. After about two weeks it reaches its limit of growth, forms spores and dries down to a somewhat thin crust. With the white mold there commonly develops, also, a considerable quantity of *Oidium lactis*, see page 55, and without much doubt this organism contributes in a measure to the resulting

product.¹ The growth of these two organisms together neutralizes the acid of the curd at the surface of the cheese and renders it slightly alkaline.

In the meantime the mold has secreted a chemical ferment, an enzyme, which has the power of digesting the curd. As fast as the acidity of the curd is reduced and the enzyme secreted, the latter acts upon the curd, changing it from a hard consistency to a soft texture. This softening begins over the whole of the cheese, immediately underneath the mold on the surface, and extends its way toward the center. At first, the cheese is a hard, solid curd from surface to center, but as this enzyme acts beneath the mold there is seen a thin layer of softened material. This layer grows deeper and deeper as it encroaches upon the curd. The enzyme produces a profound change in the casein, converting it first into peptones and similar bodies; later, these break down into still simpler bodies, or "end products," among which ammonia may always be detected. These latter end products give the flavor, and appear to be produced by bacteria rather than by the action of the enzymes secreted by the mold. During the ripening the cheese will be found to have a core of a sour, acid curd in the center, surrounded by a layer of soft, digested material. (Fig. 65.) The cheese ripens thus from the surface inward and is not completely ripened until the soft layer reaches the center. One of the difficulties in making a satisfactory cheese is to have the ripening reach the center so as to ripen the cheese completely before it becomes too soft at the outer edge—overripe. The degree of softness varies greatly. Sometimes the cheese becomes so soft that it will run out like cream when the cheese is cut, a condition that is prized by some connoisseurs. More commonly it is of a waxy or soft, buttery texture, not soft enough to "run." The waxy brands are less highly flavored and in general more popular.

¹ Mazè. *Rev. Gen. d'Lait*, iv., p. 472, 1905.

The flavors are not due to the enzyme digestion but to the end products of decomposition. In the case of this cheese, as in the hard cheeses, no positive knowledge is at hand as to the exact source of the flavor. That it is not due to the mold alone is certain from the fact that the softened cheese may be nearly tasteless, if a pure culture of mold has completed the ripening. The peculiar Camembert flavor is beyond doubt associated with some of the micro-organisms growing on or in the cheese. Bac-



FIG. 65—CAMEMBERT CHEESE, CUT OPEN TO SHOW THE SOFTENING OF THE CURD

teria commonly grow upon the surface of the cheeses, producing, late in the ripening, a red-brown color characteristic of many good cheeses; but some cheeses with an ideal flavor do not show this red color, and hence it does not appear essential. These reddish-brown areas appear chiefly where the layer of mold has been rubbed or torn away. The *Oidium lactis* is certainly concerned in the flavoring of the cheeses, at least in some cases, for if the *Oidium* is too abundant the flavor is ruined, while, if it grows moderately, the flavor is ideal. Apparently the flavor

is affected also by the type of lactic starter; for some acid organisms, when used for starters in the cheese, produce different flavors in the final product from those produced by others. All that can be safely stated at the present time is, that while the digestion is due to the mold, the flavors are the result of the action of other organisms, probably bacteria aided by *Oidium lactis*, and that various types of undesired flavors are due to the over-growth of *Oidium* or some other mischievous bacteria.

The manufacture of these cheeses in Europe has been carried on only by empirical methods; for the micro-organisms present in European dairies appear to be the ones that are needed to produce the desired ripening of the Camembert cheese. All that the cheese makers need is to make their cheeses according to a regular rule and expose them to the organisms that are natural to their dairies. Sometimes, even in Europe, these methods do not produce a sufficient inoculation of the white mold, and the cheese suffers in consequence, and when the same methods were tried in America the proper mold never appeared. In earlier years, when our cheese makers tried to adopt the same method of cheese-making, they produced a different type of cheese. The molds which grew upon the surfaces of such cheeses in our dairies were not the white mold, but either a blue mold or the *Oidium lactis*, and these produced very different results from the white species, spoiling the cheeses. Hence it was found necessary to prevent their growth. But in doing this, and not knowing that he needed to substitute the white mold, the cheese maker produced a type of cheeses different from the real Camembert, with bacteria and yeasts on its surface instead of molds. To be sure he called them Camembert, but they were really nearer a Limburger cheese, and no one who knew the imported product would fail to see a great difference between them. The real Camembert was mild and delicate; the American type strong and tasted of incipient putrefaction.

In the last few years, aided by the work of the agricultural department, an attempt has been made to introduce into this country the micro-organisms necessary for the production of the cheeses, and to inoculate them artificially into the dairies, with the hope of making on this side of the Atlantic a real Camembert cheese. The result of these experiments has shown that it is perfectly possible to make such cheeses, and many have been made that are equal in character to the imported ones. It has proved, however, to be extremely difficult to handle the organisms in a manner that is uniform and certain to produce desired results. At the present time, this industry has not been placed upon a firm footing. The cheeses are made in considerable quantities in this country at the present time, but while some of them are equal to the imported, the quality of the general product is still below that of the imported cheese. We may confidently expect, however, that success will be achieved in this line in the not distant future.

In the manufacture of the Camembert type of cheese in America the difficulties experienced are due to the undue growth of organisms that are deleterious and that produce unpleasant results. Sometimes the *Oidium lactis* grows too luxuriantly upon the cheese; when it does so it prevents the mold from getting the proper growth, and the resulting cheese has a very strong, unpleasant taste. Sometimes the class of *liquefying bacteria* get into the milk, and the lactic acid bacteria do not grow sufficiently to prevent their developing. During the period of ripening the moist curd furnishes them every opportunity to grow, and they develop bitter tastes, as we have learned they will do, if they grow too luxuriantly. In some cases, the mold develops too luxuriantly, the cheese digesting too rapidly under the rind and becoming much too soft here before the ripening reaches the center. A variety of other troubles appears in the manufacture of these cheeses, all of which are due to the improper control of the various organisms

concerned in the ripening. It will be recognized that in the manufacture of a product which requires the proper succession of three or four distinct species of micro-organisms, the problem of their control becomes extremely difficult, and it is not surprising that the artificial use of these cultures has not always

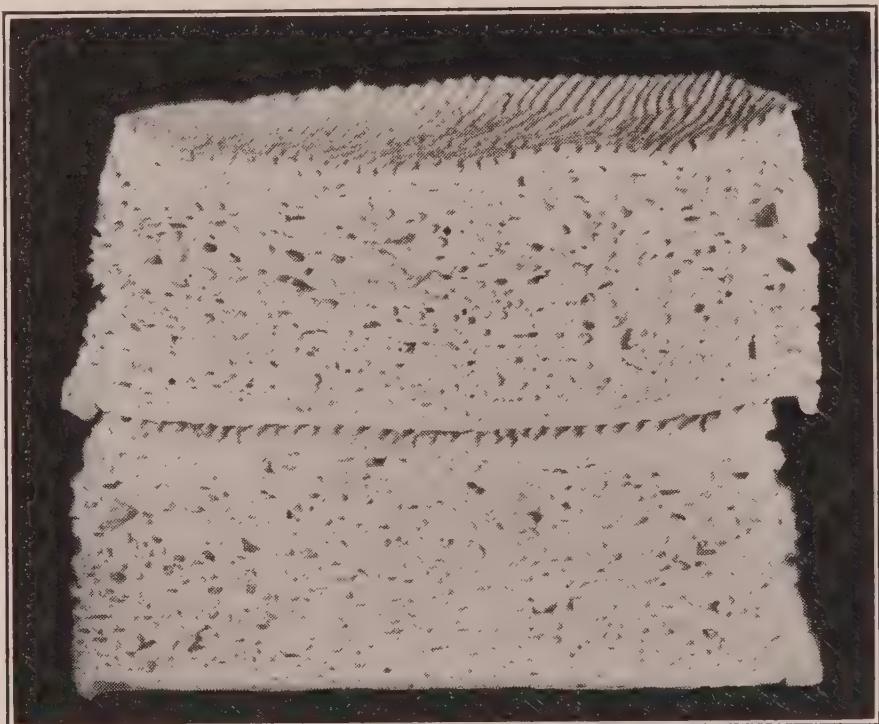


FIG. 66—WORTHLESS CAMEMBERT CHEESE
Showing porous texture produced by gas-forming bacteria

been successful. When we remember, too, that the curd is so full of moisture that it offers the very best medium for bacteria growth we can readily understand the difficulties that the maker of soft cheeses may meet. (Fig. 66.)

These various troubles must be met by different methods, but there are two general plans of action that help to meet them all. The first is that of *pasteurizing* the milk. While, as already pointed out, pasteurizing the milk does not seem to be practical with the hard cheeses, since the cheeses do not ripen,

it is perfectly feasible with the soft cheeses. By thorough pasteurizing most of the mischievous bacteria in the milk can be killed, and then, if proper lactic bacteria and mold cultures are subsequently inoculated into the milk, the ripening will take place normally and more uniformly. This procedure has been adopted in some of the better and more scientific factories in Europe, and is also being taken up in this country in the manufacture of these cheeses. The manipulation of the pasteurized milk needs to be slightly different, but offers no great difficulty. The second general safeguard for the cheese is the use of a large amount of a vigorous lactic acid starter to hold in check the growth of the organisms that would otherwise be likely to produce trouble. By these two methods it will probably be possible to control the ripening and to avoid the irregularities that have too frequently arisen.

THE ROQUEFORT TYPE

This type includes the Roquefort, the Stilton and the Gorgonzola cheeses. Their method of manufacture is somewhat different, but, in all cases, the essential character of the cheese is produced by the growth of the same species of mold *within the cheese*. They do not have a moldy rind and show no external evidence of the ripening agent. Anyone familiar with these cheeses will remember that when ripened they are filled with green masses and streaks, and microscopic study shows that these are the spores of a species of mold that has been named *Penicillium roqueforti*. (Fig. 67.) It is much like the Camembert mold, only that the latter is white, while the Roquefort mold, when it produces spores, is a blue green. In the ripening of the Roquefort, Stilton, etc., the molds do not grow on the surface, as in the case of Camembert cheese, but they grow *within the cheese*; and to bring this about the texture of the curd is made somewhat porous, so that it contains abundant air spores within, into which the mold can grow.

In the manufacture of the typical Roquefort cheese, the following general procedure is adopted. The cheese maker begins by cultivating the necessary mold upon bread; and after the moldy bread has produced a great quantity of spores, the mass is dried and ground into powder. All true Roquefort is made from the milk of the sheep, although cow's milk is sometimes used, in part, in making an inferior grade.¹ Goat's milk is not

used for these cheeses, contrary to popular impression. After curdling the milk with rennet in the usual way, and draining the curd, it is placed in a form in a thin layer, and over the top of the layer is strewn a quantity of powdered, moldy bread with its thousands of spores. Over this is placed another layer of curd, and more mold spores; and then a third layer of curd over all. The mold is thus planted within the cheese. The whole is then pressed by moderate pressure in a form. After a few days the cheese becomes hard enough to be removed from the form, and is next placed upon a machine which punches it full of holes by means of small needles. The purpose of this is to allow air to enter into the center of the cheese, thus furnishing the molds in the center with the air they need for growth. The cheese is then put into the ripening room, where the molds develop, growing partly upon the surface, but primarily within. The surface of the cheese is sometimes covered with a growth of bacteria, forming a slimy layer, and does not show any trace

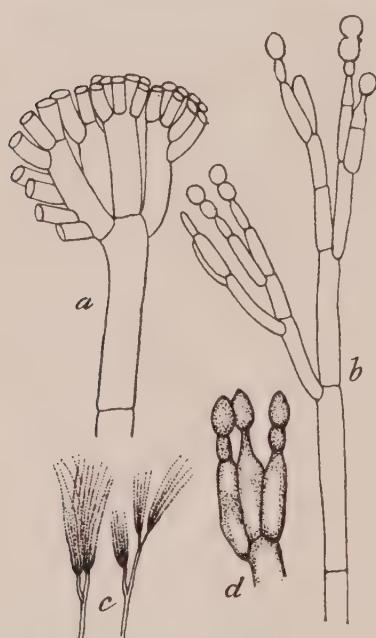


FIG. 67—*PENICILLIUM ROQUEFORTI*
Showing method of forming spores (Thom.)

holes by means of small needles. The purpose of this is to allow air to enter into the center of the cheese, thus furnishing the molds in the center with the air they need for growth. The cheese is then put into the ripening room, where the molds develop, growing partly upon the surface, but primarily within. The surface of the cheese is sometimes covered with a growth of bacteria, forming a slimy layer, and does not show any trace

¹ Milchzg., p. 152, 1903.

of the mold; but within the cheese the mold grows into the cracks and air spaces, and into the holes punched by the needles, in time permeating the whole. As the molds grow, they develop a peculiarly peppery, piquant taste, which is characteristic of the Roquefort cheese. Just before the cheese is fully ripe they taste bitter, but this taste disappears as the final flavor develops. A good Roquefort cheese is only possible when there is a luxuriant growth of these molds within the cheese, no surface growth being allowed. It is interesting to note that although the Stilton and Gorgonzola cheeses are ripened by the same mold, the method of manufacture does not commonly involve the artificial inoculation with molds, reliance being placed upon the natural inoculation with mold spores from the air or the dairy utensils. The molds grow, however, and produce a similar flavor to that of Roquefort. The makers of these cheeses sometimes punch them full of holes to stimulate mold growth. In the Gorgonzola cheeses, especially, this punching is not done till the cheeses are a month old, or more, and frequently masses of bacteria are carried by the punching needles into the holes from the surface. These may grow within the cheeses, producing discolored areas which detract from the quality of the cheese.

Most of the Roquefort cheeses are made in South France, or, at least, ripened there. The reason for this is that their proper ripening requires a low temperature and high moisture conditions, and in South France there are large, damp, limestone caves, partly natural and partly artificial, where the ripening can go on to perfection. These caves have a temperature of about 60° and not 40° , as sometimes stated. Three companies have obtained possession of these caves, and they have in their hands practically all of the Roquefort business of the world. Roquefort cheeses are sometimes made in other places, but they are practically all sent to these companies for ripening.

The manufacture of the Roquefort cheese in the United States is yet to come. There seems to be no reason why a cheese cannot be made in this country, which is ripened by the Roquefort mold and which will have the Roquefort flavor as a result; but it is not likely that a real Roquefort can ever be made in America, because, as already stated, the typical Roquefort is made of sheep's milk. It is doubtful if Americans will ever be contented to raise sheep and milk them; and unless the sheep's milk is used the true Roquefort cannot be made. Stilton cheese and Gorgonzola cheese are, however, made from cow's milk and ripened by the same mold that is found in Roquefort. These cheeses can certainly be made in this country. Stilton has already been made in Canada, and there is no reason why the manufacture cannot be undertaken and developed in the United States. It will require some experimenting and doubtless some new methods and special conditions for ripening, but there will practically be less difficulty in making this type of cheese than in making the Camembert type.

THE LIMBURGER TYPE

This type of cheese, originally produced in Germany, is now made in great quantities in this country. It is a soft cheese that is ripened wholly by the agency of bacteria, without any mold growth. No extended study of the ripening of the Limburger cheese has yet been made, but its essential nature is as follows:¹

After being drained into a form that is firm enough to handle, the cheeses are placed in a ripening cellar. Every few days they are removed from the shelves and rubbed over with some liquid, water being commonly used, although vinegar is sometimes put into the water. The surface is thus kept constantly moist. Because of this constant moisture on the surface of the

¹ Freudenberg. *Rev. Gen. d'Lait*, i., p. 477, 1902.
Roi. *Milchztg.*, p. 163, 1903.

cheese, molds cannot grow upon it, for they need a damp, not a wet surface; but a quantity of bacteria grow instead. These ripen the cheese, doubtless by the secretion of chemical ferments, although the process has not as yet been fully studied. The resulting cheese contains very high flavors, closely resembling those of decay, and the cheeses rapidly putrefy when they become old. If they are marketed at the right stage, the flavors are not strong enough to be disagreeable, and many persons are very fond of them. The *Limburger* type of cheese includes *Bachstein* and some others.

GUARDING THE MILK SUPPLY

Cheese seems more likely to be injured by unfavorable bacteria than other milk products. The reason is that it remains for such a long time, during the ripening, in a condition more or less favorable for bacteria growth. Any mischievous organisms that get into the milk have, therefore, a great chance for producing trouble before the cheese is ready for market. This is even more true of the making of soft than of hard cheeses. No cheese maker can produce good cheese from milk filled with mischievous bacteria. The cheese maker must, therefore, be on constant guard to protect his milk and all other materials from undesirable bacterial contamination. Several practical suggestions for testing and guarding milk are, therefore, especially appropriate at the close of this discussion on cheese.

Since a cheese factory is likely to receive milk from many different farms it will happen that the milk from the different sources will not be alike. It may be that while most of the milk is good, that from a single farm contains a quantity of mischievous organisms. If this milk is mixed with the rest, it may spoil the whole; but if the infected dairy can be detected, and its milk excluded from the rest until improved, the whole output will benefit. To enable the cheese maker to do this there have been devised the *Fermentation* and the *Curd Tests*.

The Fermentation Test.—This consists simply in taking small samples of milk from the different dairies, putting them in separate vials or bottles, placing them in a warm temperature, about 90°, for a few hours, and carefully examining the results. If the milk curdles in the normal fashion, with a clean smooth curd, the sample may be regarded as satisfactory. If large numbers of gas bubbles appear in any sample of milk, or if exceptionally unpleasant odors arise, it may be assumed that the milk in question contains something that is liable to produce trouble, and it will be wise, therefore, to refrain from mixing the milk from the individual patron with the general day's collection. Under these circumstances, a visit to the creamery may soon show the source of trouble and suggest a remedy.¹

The Curd Test.—The curd test is similar in its purpose, and is designed primarily to prevent gassy cheese.² It consists in placing in clean vessels—fruit cans will serve perfectly well for the purpose—about a quart of the milk of each patron. This is heated to 90°, and about ten drops of rennet added to it, after which it is allowed to stand until it curdles. The curd is cut, and the whey poured off occasionally, the whole curd being allowed finally to mat together. For this purpose it should be kept at a temperature of about 98°. After it has become well matted, the curd is cut open and examined. In the case of good milk, the curd will be fairly smooth, showing only occasional mechanical holes. If, however, the curd proves to be full of pores, indicating the presence of gas within the curd, it is practically certain that the milk contains some of the gas-producing organisms, which are liable to give rise to trouble. Under these circumstances, the milk of the patron in question should be looked upon with suspicion.

These tests are used quite commonly in some cheese-making countries, but have not, as yet, a very wide application. There

¹ Dugelli. Cent. f. Bact., II., xvii., p. 37, 1907.

² Babcock, Russell and Decker. 12th Ann. Rep. Wis. Exper. Sta., p. 148, 1895.

are some instances where they may be very useful. In the ordinary working of a cheese factory it is hardly possible to use the tests for the milk of any particular day, for it is impractical to wait long enough to test the different samples of milk before they are mixed together in a general curdling vat. But the tests may be very useful in detecting whether the milk from any one farm is constantly "off," so as to be a danger to the general supply. For tracing the source of trouble the tests may thus be of great value.

The cheese maker should constantly bear in mind the danger of contamination of his milk. Sometimes "off" flavors in cheeses have been traced to bacteria in the dairy water supply. Old barrels of souring whey are a source of trouble. The practice of soaking rennet in sour whey to extract the rennet material is sometimes common, but it is a bad practice, for such a mixture furnishes a most excellent medium for bacteria growth, and troublesome yeasts and bacteria have been traced to such a source. Dirty cans sent back to the farm without sterilization, slovenly methods in the barn, and, in short, all of the sources of bacterial contamination previously mentioned are very likely to trouble the cheese maker as much, if not more, than any others connected with the dairy industry.

PART II

LABORATORY DIRECTIONS FOR EXPERI-
MENTS IN DAIRY BACTERIOLOGY

LABORATORY WORK IN DAIRY BACTERIOLOGY

It is expected that the experiments in this section shall accompany the study of the text. Recognizing, however, that different classes of students will have different times and conveniences for laboratory work, the two parts of the subject have been separated. It must be emphasized, however, that the practical work is fully as important as the theoretical discussion, and no one can really understand the latter without the former. The course of experiments outlined will probably be too long for short courses. For this reason the important experiments that should be performed without fail are indicated by *. These are fundamental. The others may be taken as time permits. It will frequently be a good plan to divide the experiments not thus marked among different sections of the class, having each section report the purpose, method and result of such experiments to the whole class.

The directions for these experiments are given in sufficient detail, so that anyone with a fair knowledge of laboratory methods can follow them without other instruction. The private student of dairying may, therefore, carry out this laboratory course by himself, although, of course, it will take more time in this case than if adequate personal instruction were obtained.

Special emphasis should be placed upon the necessity of carefully **LABELING** every culture made and recording in a note book each experiment, its purpose and results.

It may not always be possible for the student to follow the *order* of experiments given. There is no necessity for following the order given except that numbers 1 to 13 should precede all others.



FIG. 68—TEST TUBES—BOARD OF PLATINUM WIRE TO BE FUSED INTO HEALTH PATTERN GLASS RODS.

Common tumblers are frequently more convenient than test tube racks for holding test tubes.

Culture oven, with constant temperature of 98°. This is expensive and may be omitted.

LIST OF APPARATUS NEEDED

Steam sterilizer.

Autoclav.

Hot air sterilizer. A common gas oven used for cooking will do.

Stew pan for cooking media.

Flasks—1 liter and 1½ liter.

Test tubes—Board of Health pattern.

Petri dishes—4 inches in diameter.

Pipettes—1 c.c., 2 c.c. A few larger ones are also convenient. Each of the smaller pipettes should have a glass tube to contain it, sealed at one end.

Wire baskets to hold test tubes.

Test tube racks.

Burette holder and 4 burettes.

Evaporating dishes.

Measuring cylinders—1 liter and 100 c.c.

Counting plate or counting card.

Platinum wire to be fused into glass rods.

Bunsen burners

Forceps—Common and Cornet forceps.

Microscope, including a $\frac{1}{12}$ -inch immersion lens and plenty of slides and cover glasses.

A counting plate or counting cards.

MATERIALS

Peptone. Phenolphthalein.

Salt. Alcohol.

Beef extract. Corrosive sublimate.

Gelatin—gold label. NaOH.

Agar Agar. HCl.

Litmus, dry in cubes. Immersion oil.

Absorbent cotton. Methylen blue.

Common cotton, good quality.

Normal NaOH } These solutions will best be bought from dealers. They may be made by methods described in No. 2 and 3, but the beginner will hardly be able to make the Normal HCl successfully.

Normal HCl }

Dextrose, lactose and saccharose.

PRACTICAL WORK¹

***No. 1. Washing Glassware.** All glassware used in bacteriological work must be thoroughly washed. No special directions need be given save that hot water and soap are necessary. New glassware should be treated first with 1% HCl. Used glassware that contains the remains of gelatin or other media must first be boiled in water, preferably containing a little sal

¹ The following laboratory manuals may be found useful as books of reference:

Moore. Laboratory Directions for Beginners in Bacteriology. Ginn & Co., 1900.

Frost. A Laboratory Guide in Elementary Bacteriology. The Macmillan Co., 1903.

Gorham. A Laboratory Course in Bacteriology. W. B. Saunders, 1901.

Van Slyke. Methods of Testing Milk and Its Products. Orange Judd Pub. Co., 1906.

soda or powdered soap. After boiling, wash well in hot water and then rinse thoroughly in clear cold water; drain and allow to dry.

The student may begin by thus washing 50 test tubes (Board of Health pattern, Fig. 68), 2 liter flasks, 1 dozen Petri dishes and several 1 c.c. pipettes. After drying, plug the test tubes and



FIG. 69—PIPETTE AND GLASS TUBE TO HOLD SAME WHEN STERILIZED.

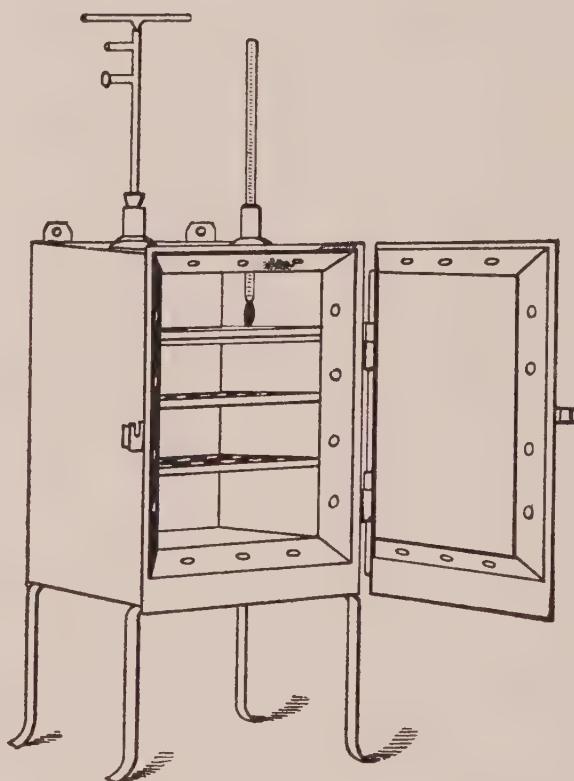


FIG. 70—HOT AIR STERILIZER

flasks with cotton, place the pipettes in pieces of glass tubing closed at one end and plugged with cotton at the other (Fig. 69). Place all the articles in the sterilized oven (Fig. 70), and heat to 155° C. (310° F.) for one hour. This sterilizes them and

they are ready for use. All glassware subsequently used should be treated in the same way.

***No. 2. Normal NaOH Solution.** To make this with absolute accuracy special chemical methods are necessary. A solution which is nearly accurate, sufficiently so for all purposes of this work, can be made by dissolving 40 grams of pure NaOH (in sticks) in one liter of water. This should be kept in a tightly closed bottle. It does not maintain its strength long and should frequently be made fresh. To make $1/10$ normal NaOH, one part of normal is mixed with nine parts of distilled water.

***No. 3. Normal HCl.** This is more difficult to make, and it will usually be best to buy it, already made, of dealers in chemical supplies. It can be made as follows: Add 50 c.c. of chemically pure HCl to 450 c.c. of distilled water. If it is of the right strength, one c.c. of it will exactly neutralize one c.c. of normal NaOH. Place one c.c. in an evaporating dish, dilute with 10 times its bulk in water, add a few drops of phenolphthalein, and then exactly one c.c. of normal NaOH. If the mixture turns red, it means that there is not enough acid in the solution, and a little more should be added. If no red appears, add a drop or two more of $1/10$ normal NaOH, and see if the red color appears. If it does, the HCl solution may be regarded as normal. If, however, it requires several drops to produce the red color, the HCl solution is too strong and must be weakened by adding water. By adding a little acid or water as may be indicated, and testing repeatedly, the HCl solution may be brought to a strength where it will exactly neutralize the same amount of NaOH. When this point is reached, it is to be labeled Normal HCl.

***No. 4. Preparation of Bouillon.** Measure out the following:

Water	1 liter
Liebigs extract of beef	3 grams
Common salt	5 grams
Peptone	10 grams

Place in some dish in which the mixture may be boiled, and carefully weigh the dish and its contents. Dissolve the mixture at a temperature of about 150° F. (60° C.), after which weigh again and restore the evaporated water by bringing the weight to its original mark.

Bacteria culture media need to have a proper grade of *acidity*. If the amount of acid is either above or below the desired point the bacteria either do not grow at all or not very readily. The method of bringing the bouillon to the desired point is somewhat complicated and must be described in detail.

a. Measure out five c.c. of the bouillon, place it in an evaporating dish with ten times its bulk of water, and bring nearly to a boil. While still hot add to it a few drops of phenolphthalein.¹ This is a solution which is colorless so long as it is acid, but turns red as soon as it becomes alkaline. The bouillon is always acid and consequently the phenolphthalein will make no change in its appearance.

b. Place the evaporating dish under a burette containing 1/10 normal NaOH. Take the reading of this solution carefully in the burette. Allow the solution to drop from the burette into the evaporating dish, drop by drop, stirring carefully. (See Fig. 60.) As long as the material remains acid no color will appear. Allow the solution to drop into the dish until a faint red color makes its appearance and does not disappear upon stirring. When this point has been reached the contents of the evaporating dish are at a *neutral point*.

c. Taking the reading for the burette, determine how many cubic centimeters of the solution have been used to neutralize the 5 c.c. of bouillon. Divide by 5 to determine the amount necessary to neutralize 1 c.c. and multiply by 995 to determine the amount necessary to neutralize the rest of the bouillon.

d. Add to the entire quantity of bouillon enough NaOH to neutralize it, but in doing so use normal NaOH instead of 1/10

¹ 8 per cent. dry phenolphthalein in 50 per cent. alcohol.

normal. It will, of course, require only $1/10$ as many cubic centimeters, as calculation has shown above would be required of $1/10$ normal. This will bring the culture media to the neutral point.

e. This medium is now not acid enough for the best bacteria growth. The best reaction is one in which there is 15 cubic centimeters of normal HCl to each liter of the media (1.5%). Consequently to this bouillon there should be added 14.9 c.c. of normal HCl which will give the desired acidity, 1.5%.

An example will show this more clearly. Suppose that it required 1.5 cubic centimeters of the $1/10$ normal NaOH to neutralize 5 c.c. of the bouillon. Dividing this by five would give $.3$ c.c. required to neutralize 1 c.c. Multiplying by 995 would give 298 c.c. required for neutralizing a liter. Dividing by ten to reduce this to normal NaOH will give 29.8 c.c. Therefore, 29.8 c.c. normal NaOH is added to the bouillon, giving the neutralizing point. After this, 14.9 c.c. of normal HCl added to the material will give an acidity, 1.5%.

f. After obtaining the desired acidity, bring the bouillon to a brisk boil for 15 minutes, after which enough water must be added to restore to the original weight. Heat once more to boiling and filter through filter paper into a sterile flask. Place about 10 c.c. in a few test tubes. Replace the cotton plug in both the flask and test tubes; place the test tubes and the flask in a steam sterilizer and steam for 20 minutes (Fig. 71). Put aside at a temperature of the room for 24 hours, then steam



FIG. 71—STEAM STERILIZER

again for 20 minutes. Set aside another 24 hours and steam again. The bouillon is now sterile and can be kept indefinitely.

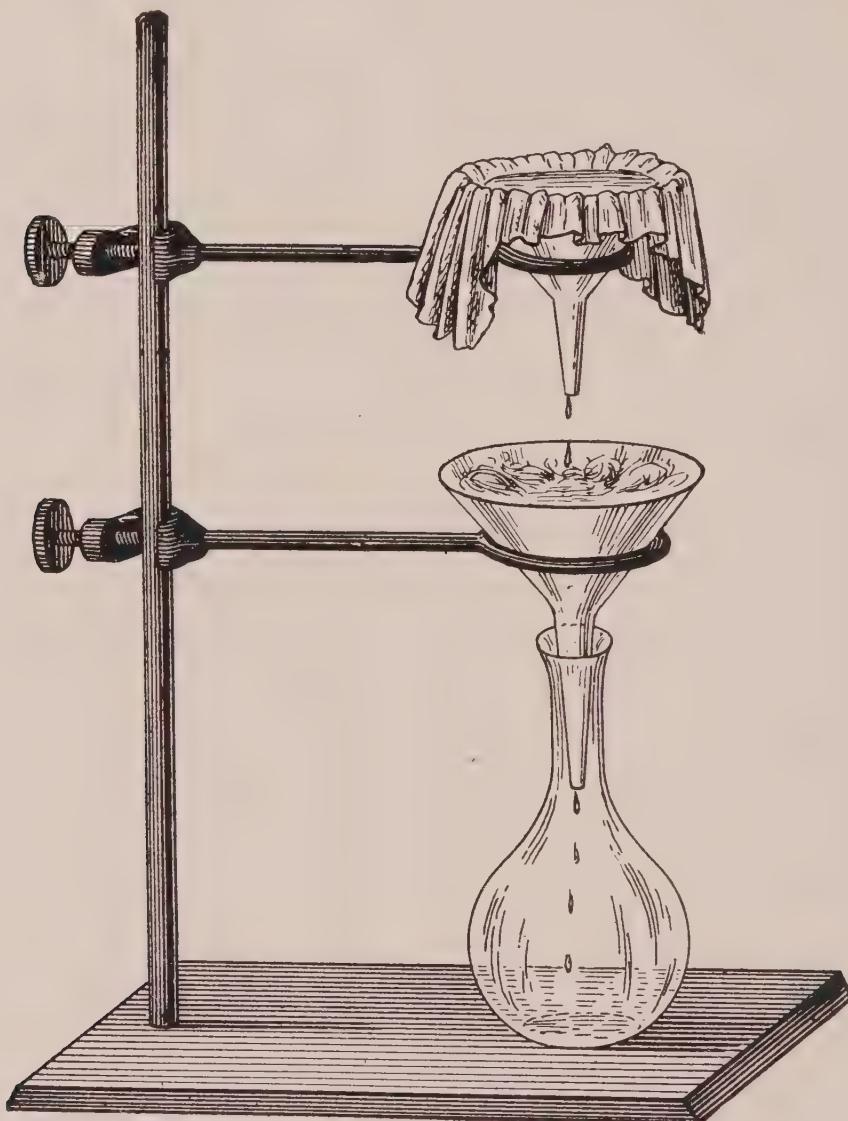


FIG. 72—FILTERING CULTURE MEDIA THROUGH ABSORBENT COTTON
The upper funnel containing cheese cloth, the lower absorbent cotton

If the bouillon or any part of it is to be made into agar or gelatin as shown below, the neutralization and sterilization may be omitted.

***No. 5. Agar Culture Medium.**

a. To 500 c.c. bouillon prepared as in (3) add 1.5% of agar

(7.5 gm.). Allow the agar to soak in the cool bouillon for $\frac{1}{2}$ hour. Place in a stew pan and record the exact weight of

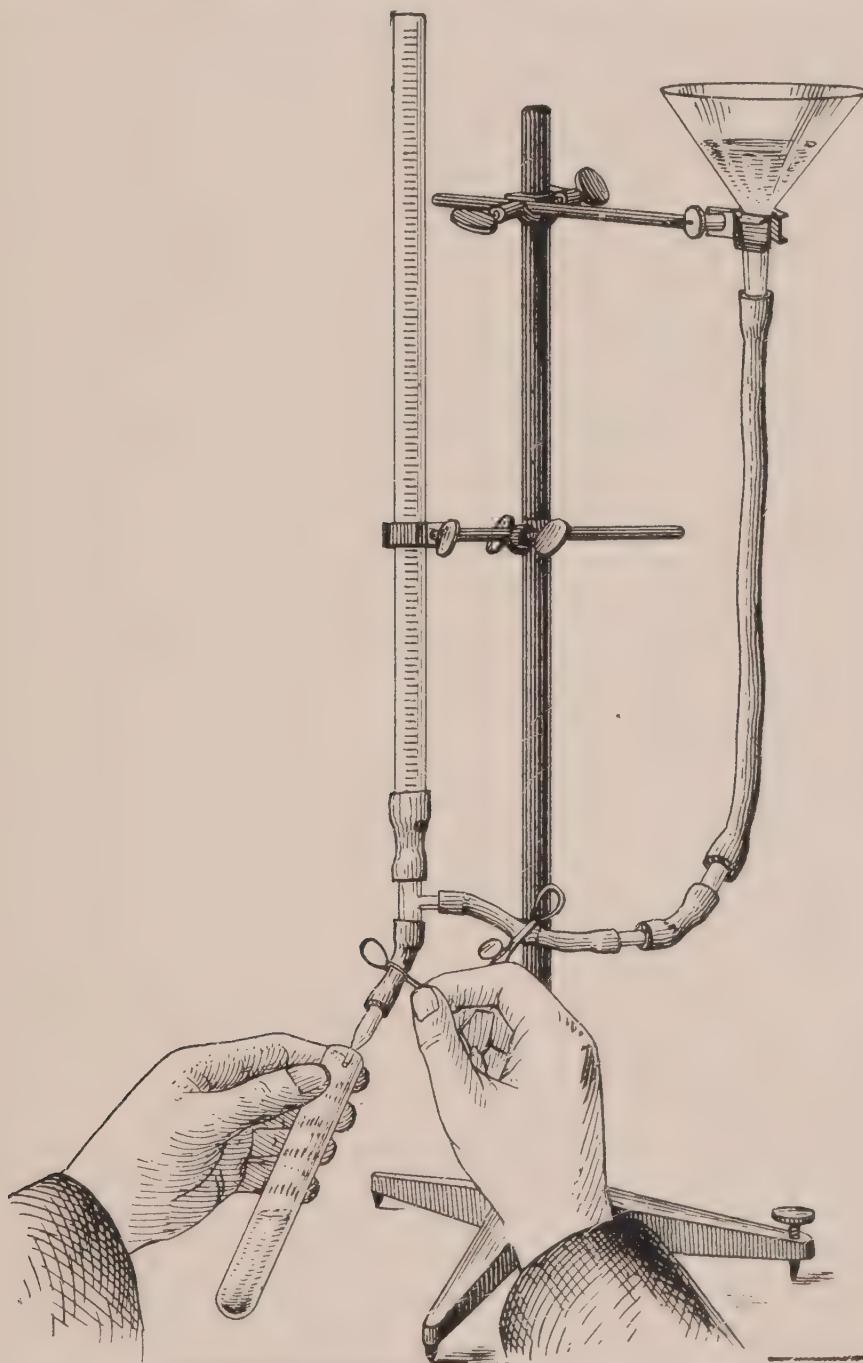


FIG. 73—FILLING TEST TUBES WITH A MEASURED AMOUNT
OF A CULTURE MEDIUM

dish and contents. Boil until all the agar is dissolved. Weigh again and restore to original weight by adding water.

b. Determine acidity. Neutralize and adjust reaction to 1.5% HCl, as described in 4, *c.*

c. After obtaining the proper acidity bring the mass to a brisk boil, and while it is boiling add to it the white of an egg, previously mixed with a little water. Boil hard for 20 minutes.

d. Weigh, and add water to bring it back to its original weight. Bring to a boil.

e. While boiling, as in *d.* place in a large funnel a considerable quantity of absorbent cotton. Over the top of the funnel, or in a second funnel over the first, place some cheese cloth (Fig. 72). Pour the hot agar mass through the cheese cloth into the absorbent cotton. It will run through the cotton and may be caught below in a flask. It should be transparent, clear, and of a light yellowish-brown color.

f. From the flask fill a number of sterilized test tubes, putting about 10 c.c. into each. (Fig. 73.) Replace the cotton plug. In filling these tubes it is best to use a funnel, and care should be taken not to allow the agar to touch the tube where the cotton is afterwards to be inserted, as it will stick to the cotton.

g. Place the tubes, together with what remains in the flask, in a steam sterilizer, figure 71, and allow the steam to act on them for 20 minutes. Then remove and set aside in the temperature of an ordinary room.

h. 24 hours later put the material again in a steam sterilizer, and sterilize for $\frac{1}{2}$ hour.

i. 24 hours later sterilize again in steam for $\frac{1}{2}$ hour. The material is now finished and may be preserved for future use.

j. Agar slants. After the last steaming, while the agar in the tubes is still hot and liquid, lay 20 of them down on a table with their mouths slightly raised so that the agar will form a slanting surface in the tubes. (Fig. 74.) Allow them to harden in this position.

***No. 6. Determination of the Number of Bacteria in Milk.**

It is always first necessary to dilute the milk with sterilized water, since ordinary milk contains so many bacteria that, unless diluted, the numbers will be too large to be estimated properly. Milk contains such a widely varied number, however, that it is difficult to determine beforehand how much to dilute the material; and one must be guided, when possible, by knowledge as to the age of the milk. Where a miscellaneous lot of milk

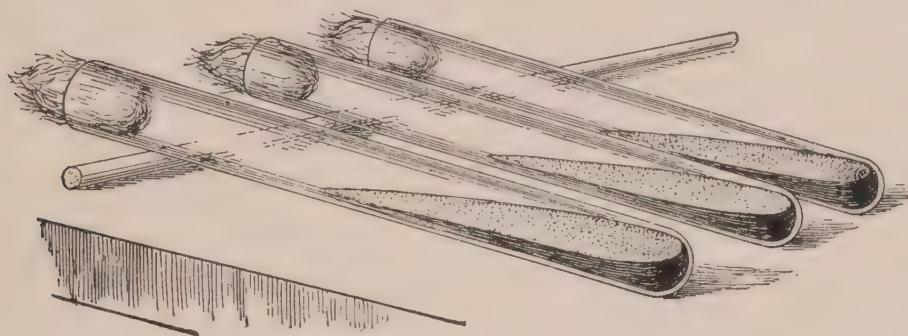


FIG. 74—HARDENING AGAR SLANTS

is to be studied for this purpose, probably the best method is to dilute it 1,000 times.

In order to dilute milk 1,000 or any other definite number of times, it is necessary to have flasks and vials measured to hold certain definite amounts. It is most convenient to have some holding 4 c.c., 9 c.c., 19 c.c. and 99 c.c. (Fig. 75.) From them the desired dilution can readily be obtained.

a. Fill a number of these vials to the mark with water, plug with cotton and sterilize. This can best be done in an autoclav. (Fig. 76.) In using this autoclav place a small amount of water within it and then the vials to be sterilized. Replace the cover, fastening it down tightly with the screws designed for the purpose, and light the gas. Leave the cock open until steam begins to come out, and then close. Allow the steam pressure to rise to 15 lbs. and maintain this pressure for one hour. Allow the autoclav to cool before opening. The vials may then be

removed ready for use and, since they are sterile they will keep indefinitely. They will be called *water blanks*. (Note.—The autoclav may be used to sterilize other liquid media. If it is used for gelatin care must be taken not to allow the pressure

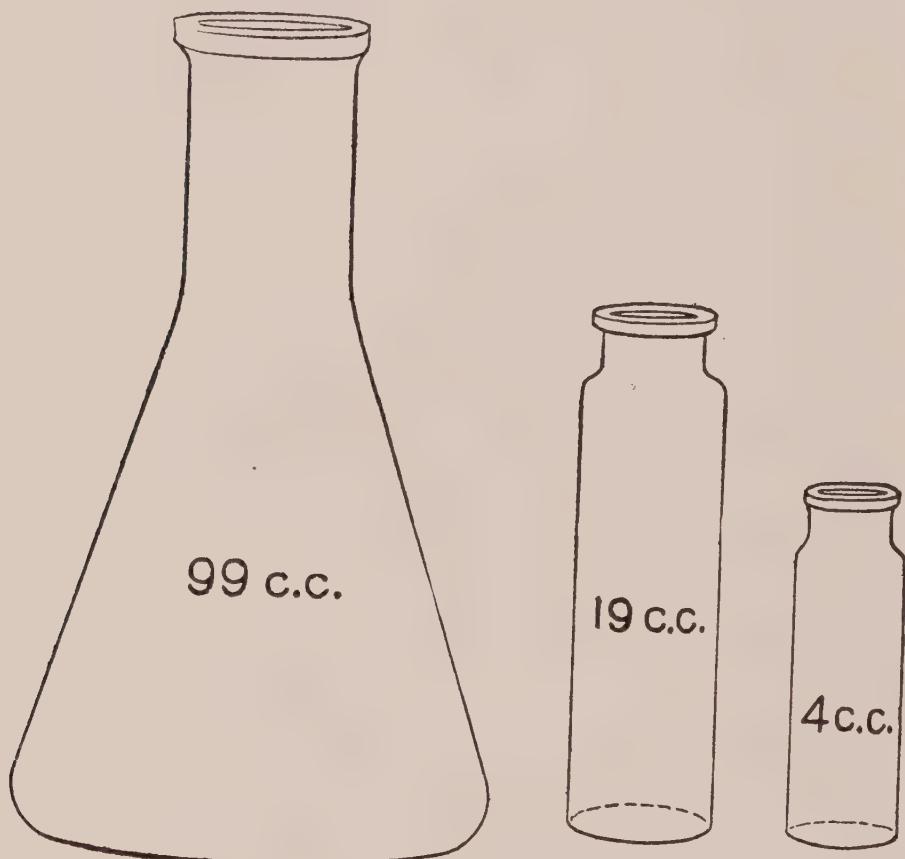


FIG. 75—VIALS FOR MILK DILUTION

to rise above 5 lbs., or the gelatin will refuse to harden subsequently. The successful use of the autoclav for sterilizing gelatin requires considerable experience.)

b. Shake the milk to be tested, very vigorously, in order to insure uniformity, and, with a sterile pipette remove one c.c. to one of the vials holding 9 c.c. of water. Shake, and with a fresh pipette transfer one c.c. to a second water blank of nine c.c. Shake again, and with a fresh pipette transfer one c.c.

to a third similar water blank. This last vial will contain $1/100$ of a c.c. of milk and one c.c. of it will contain $1/1,000$ of a c.c.

It will readily be seen that any modification of the dilution may easily be made. If two water blanks only are used, the dilution will be 100. If four are used, it will be 10,000. If very high dilutions are desirable, for example, $1,000,000$, six vials can be used, or it is better to proceed somewhat differently. Place 99 c.c. of water in each of three flasks or bottles, and sterilize. To one add 1 c.c. of the milk to be tested, and, after a thorough shaking, transfer 1 c.c. into a second flask containing 99 c.c. Transfer 1 c.c. of this to a third flask and 1 c.c. of this last will contain $1/1,000,000$ of a c.c. of milk. By very simple calculation any desired dilution may be obtained.

c. Melt four test tubes of the agar medium in hot water and cool to a temperature of about 50° . Into each tube place, with a sterilized pipette, 1 c.c. of the diluted milk and then mix thoroughly by *gentle* shaking. After the mixing pour the contents of the tube into a sterilized petri dish (Fig. 77) and cover immediately. As soon as the agar hardens place the petri dish, now called an *agar plate*, in an incubating oven at a temperature of 37° . It is always well to make at least four such plates in order that an average may be taken for the final result, since no one plate can be relied upon for very great accuracy. After 24 hours at 37° examine the plates; they will be covered with

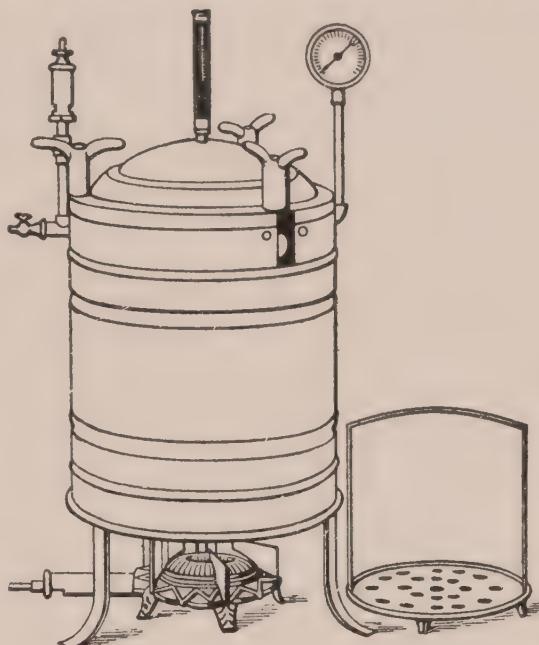


FIG. 76—AUTOCLAV, FOR STERILIZING
UNDER STEAM PRESSURE

small dots, each of which represents what is called a *colony* of bacteria. They are supposed in all cases to be a mass of bacteria that have developed from a single individual in the original milk which has been fixed in its position by the hardened agar. If every bacterium in the original milk has thus grown and produced a colony, it is only necessary to count the colonies

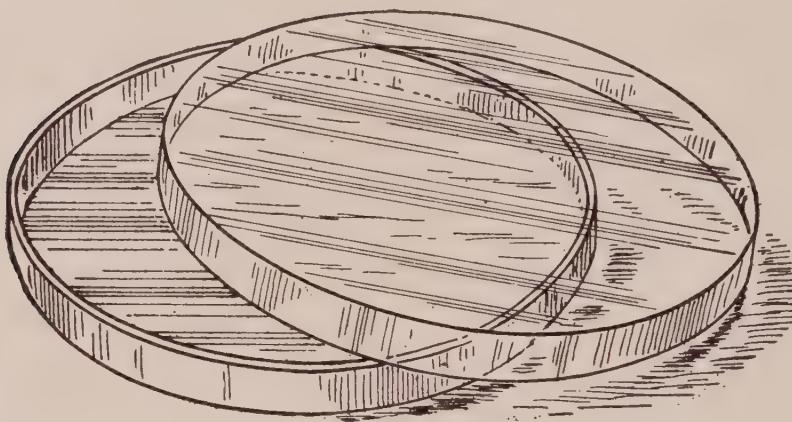


FIG. 77—PETRI DISH

in each agar plate to find the number of bacteria in $1/1,000$ of the original c.c. of milk product, which was the amount taken.

Count the numbers on each plate; add them together and divide by 4 to determine the average number. Multiply by 1,000 to give the number of bacteria per c.c. of the milk.

For convenience in counting, the bottom of the plate may be divided into small areas by marking with a wax pencil. If the number is very great use a counting plate or counting cards. These are divided into areas of 1 sq. centimeters. Place the plate over the card and count the number of colonies on each of several of the 1 cm. areas and obtain the average. Determine by the card the number of such areas in the whole plate, and by multiplying calculate the total number of colonies on the plate.

The box shown in figure 78 will be found very convenient in counting the colonies. It consists of a shielded lens, and a slate

base with a divided circle. It is made by the International Scientific Instrument Co.

It will be evident that the number thus obtained will always be an under estimate of the number of bacteria, because some bacteria will not grow in the agar culture medium. There are also other facts which decrease the number of colonies, so that the numbers thus obtained will never be equal to the total number of bacteria.

*No. 7. Gelatin Culture Media.

a. Measure out and place in a stew pan a definite quantity of the bouillon prepared as above described and add to it 12% of a first grade gelatin, "Gold Label Gelatin" being preferred. Allow the gelatin to soak in the bouillon till soft and almost melted. Weigh the whole material, with its containing dish, and record weight. Boil briskly for five minutes, then add water to restore original weight.

b. Determine acidity and bring reaction to 1.5% HCl, as described in *2, b* to *e*.

c. Boil 15 minutes. Add the white of an egg and boil briskly for 15 minutes. Replace the water of evaporation.

d. Heat once more to boiling and filter through absorber^t cotton, as illustrated in Fig. 72.

e. Fill 25 test tubes with about 10 c.c. each; sterilize the tubes and the rest of the gelatin in a flask, by steaming in

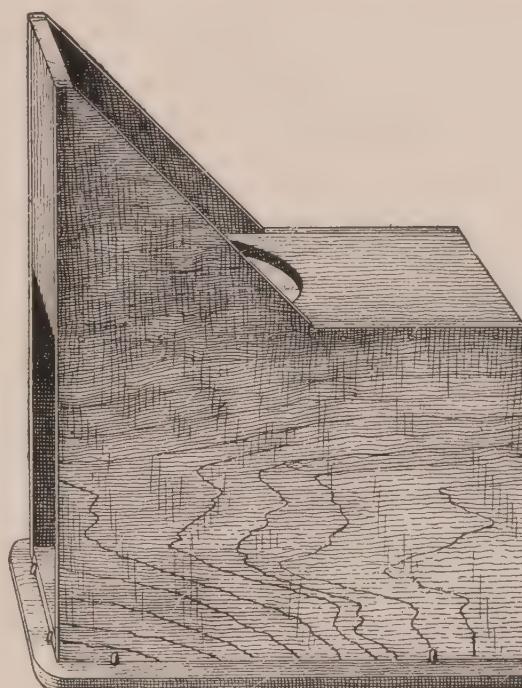


FIG. 78—COUNTING-BOX
(International Instrument Co.)

steam sterilizer 20 minutes. Allow to stand 24 hours in room temperature, and steam again for 20 minutes. 24 hours later steam a third time. It is now sterile and may be reserved for future use.

***No. 8. Litmus Gelatin.** For the study of milk bacteria it is very desirable in most cases to detect the production of acid. For this purpose a litmus culture medium is used, the preparation of which is as follows:

a. To 500 c.c. bouillon add 15% gelatin and 1% lactose (milk sugar). The rest of the process up to the point of filling the test tubes is exactly as above described in 5, *a* to *e*. In filling test tubes, *exactly* 8 c.c. of the medium is to be placed in each of the test tubes, and the test tubes are to be sterilized on three successive days.

b. Prepare a litmus solution by weighing out 50 grams of solid litmus (the litmus cubes preferred). Soak for 24 hours in 5 times its weight of water; filter through filter paper, and a deep blue solution will be obtained. This is too alkaline, and it is necessary to bring its reaction to the same point as the reaction of the gelatin—namely, 1.5%, as follows:

c. Measure out 5 c.c. of the litmus solution in an evaporating dish; dilute with 10 times its bulk of water, and heat. Add to it from the burette 1/10 normal HCl, drop by drop, until the blue turns from a deep blue to a faint red. This gives the *neutral point of litmus*.

d. By the reading on the burette before and after neutralization determine the number of c.c. required to neutralize 5 c.c. of the litmus. If we divide by 5 to give the amount required to neutralize one c.c. and multiply by 495, we shall have the number of cubic centimeters necessary to neutralize the whole litmus solution. If this amount of acid is added it will bring the litmus solution to the litmus neutral point, which is different from the phenolphthalein neutral point used in 4, *b*. The litmus neutral point is 2.5% more acid than the phenolphthalein neutral

point, and it is desired to make the reaction 1.5%. To do this several methods are possible, the simplest of which is as follows: To the litmus solution add the amount of HCl necessary to bring it to the phenolphthalein neutral point, and then add 10 c.c. per liter of normal NaOH to bring it back to 1.5%.

An actual example will make this clear. Suppose we start with 500 c.c. and find that it requires 1.5 c.c., 1/10 normal HCl to neutralize 5 c.c.

$1.5 \div 5 = .3 \times 495 = 148.5$ c.c. 1/10 normal required for 495 c.c. of solution.

$148.5 \div 10 = 14.8$ c.c. normal HCl. Add 14.8 c.c. to the litmus solution.

$.495 \times 10 = 4.9$. Hence, by adding 4.9 c.c. normal NaOH, the litmus solution will be brought to the desired reaction, 1.5% acid. Since this solution and the gelatin made in *a* are of the same degree of acidity, they may be mixed in any proportion without changing the reaction.

c. Sterilize the litmus solution in the same way as the gelatin. The method of combining the gelatin and litmus will be mentioned below.

No. 9. Litmus Agar. This is prepared by adding to ordinary agar (5) 1% milk sugar and enough litmus solution to give it a blue color. It has some advantages over plain agar, since it differentiates acid colonies. It is not so useful as litmus gelatin.

***No. 10. Gelatin Plates.** Gelatin culture media offer a number of advantages over agar, since they enable us to tell more satisfactorily the kinds of bacteria which may be present in the milk. It is more difficult to use, however, and results more frequently in failure, for reasons to be noted presently.

In making gelatin plates from milk, it is desirable to dilute the milk to such an extent that the number of bacteria in each plate shall be not more than 500 or 600, preferably not more than 200. If the numbers are higher than these, there is likely to be trouble and inaccurate determinations. For this reason,

to make a successful plate with gelatin, it is necessary to know something of the age of the milk, in order that the proper dilution may be obtained. If milk is not more than 24 hours old, and has been kept fairly cool, a dilution of 1,000 will ordinarily be satisfactory; sometimes a higher dilution is necessary. This difficulty of determining the amount of dilution is the difficult point of the gelatin method. For this reason, when gelatin is used for estimating bacteria in milk, it is desirable to make several plates of different dilutions in order that the proper one may be reached in one at least.

Procure some milk from 12 to 24 hours old. Dilute it as above described so that one vial has a dilution of 1,000, another of 10,000, and another of 100,000. Add 1 c.c. of each dilution to separate tubes of the melted gelatin, thoroughly mix and pour into the Petri dishes, replacing the cover. It is best to make at least two such Petri dishes from each solution. Place at first in a cool place to harden. The plates must be kept in a temperature of not much over 70° or the gelatin will melt. For this reason it is difficult to use gelatin in summer. The bacteria at 70° do not grow as rapidly as they do at 37°, and the colonies do not begin to appear, usually, for 2 days. The appearance of the colonies in the gelatin plates is much different from that in agar plates, as will be noticed later.

***No. 11. Litmus Gelatin Plates.** This requires only a slight modification of the process described in the last paragraph. Dilute the milk as above. To several tubes of gelatin prepared in 8, *a*, add 2 c.c. of the litmus solution prepared in 8, *b* to *c*. Melt and mix thoroughly. Place in each tube 1 c.c. of the diluted milk, then pour into the Petri dishes, after hardening, keep at about 70°. After the colonies have grown for two or three days, those which produce acid will be found to be red, or surrounded by a little red halo where the blue litmus has been turned to red. It is, therefore, easy by examination to determine the number of acid producing bacteria in such milk.

From the fact above stated it will be evident that for a general determination of the number of bacteria in samples of milk whose origin and age are unknown, it is easier to use agar with a dilution of 1,000 than gelatin. If we have an approximate idea of the number of bacteria, so that the proper dilution may be determined, the use of litmus gelatin will enable us not only to determine in numbers, but will give the means of fairly well separating the different kinds of bacteria. Gelatin, therefore, will give most of the information that agar gives, and considerably more under conditions where gelatin can satisfactorily be used. The numbers obtained from plain gelatin plates are usually higher than those obtained from litmus gelatin, and the numbers from either plain gelatin or litmus gelatin are much higher than the numbers from agar.

Determine the numbers of colonies in both the plain gelatin plates and the litmus gelatin plates. Isolate several colonies as in the following:

***No. 12. Isolation of Colonies.** The bacteria which develop on plates prepared as above described, produce clusters, as already mentioned, called *colonies*. In many cases it is possible to tell the type of bacteria from the shape and appearance of the colony. A study of the plates, especially the litmus gelatin plates, will, therefore, frequently enable us to determine, not only the numbers, but something concerning the kinds of bacteria. The bacteria are now to be *isolated* from the plates. Sterilize a platinum needle (Fig. 79) in a flame, then dip the tip of it into one of the colonies. When it is removed, a number of the bacteria will adhere to it, and these can then be transferred to one of the slant agar tubes, by removing the cotton plug and drawing the tip of the wire gently over the surface of the agar. Sterilize the needle again before laying it down. Label the tube by some number, recording in a note book the appearance of the colony, and its source.

***No. 13. Purification of Cultures.** Presumably the culture

made in No. 12 will be a pure culture. By this term is meant that it will contain only one kind of bacteria. If the colony on the plate comes from a single bacterium, as has been assumed, the colony of bacteria will contain a single kind of organism. It frequently happens, however, that a single colony may come from two bacteria, side by side, in which case it may not be a pure culture. In order to be sure of having a pure culture, it is always necessary to *purify* it, as follows:

a. Fill some test tubes with about 10 c.c. of water, and sterilize one hour in an autoclav. These are *water blanks*.

b. After the cultures of No. 12 have grown from one to two days, remove with a platinum needle a small amount of the bacterial growth and transfer it to a water blank. Shake thoroughly so as to distribute the bacteria uniformly. Transfer with a platinum loop, two loopfuls of this to a second water blank, for the purpose of more highly diluting the bacteria. Again after a thorough shaking, transfer two loopfuls of this second tube to a tube of melted gelatin. Shake gently, to distribute the drop of water uniformly through the gelatin, and pour out into a Petri dish. In about two days the plate will be dotted with colonies. Pick out one with a platinum needle, inoculate upon an agar slant, and it will almost invariably be pure. This purification of cultures must be done in all subsequent work.

If it is not done, errors are sure to result from the mixture of different kinds of bacteria together, and all the work will be rendered worthless.

***No. 14. Preservation of Cultures.** After having once been planted on the surface of agar slant, agar culture may be kept alive, as long as the agar remains moist; and it may be studied



FIG. 79
PLATINUM
NEEDLES

at any time. But when the agar becomes dry, the bacteria usually die. If, however, the tube be sealed with parafin or sealing wax, it can be kept for months, in many cases for years, without dying. After purifying the cultures, carefully label, and reserve them for future use.

***No. 15. Microscopic Study.** Prepare one or both of the following staining solutions:

Methylene Blue

Saturated alcoholic solution of Methylene Blue 15 c.c.
Potassium Hydrate (1:10,000)¹ 50 c.c.

Fuchsin Solution

Saturated alcoholic solution of Fuchsin 5 c.c.
5% solution of carbolic acid 45 c.c.

Place an ordinary cover-glass in a special pair of forceps (Fig. 80) and in the center place a very small drop of sterilized

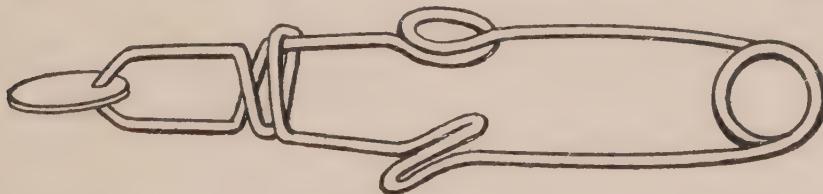


FIG. 80—FORCEPS FOR HOLDING COVER-GLASSES WHILE STAINING

or boiled water. With a platinum needle remove a very small quantity of the bacteria growth from the surface of an agar tube of No. 14 and place in the water drop. Stir this drop with the platinum wire, to distribute the bacteria, and spread as widely as possible over the cover-glass. Allow to stand in the air until dry, and then pass the cover-glass through a gas flame quickly three times. The purpose of this is to fasten or fix the bacteria firmly to the glass so that they will not wash away. It is necessary to avoid using too much heat, for over-heated

¹ To make this solution add 1 c.c. of a 10 per cent. KOH solution to 99 c.c. of water, and then add 5 c.c. of this to 45 c.c. of water.

bacteria will not stain. Place on the cover-glass enough of one of the staining solutions to cover it completely, and allow the specimen to stand undisturbed for several minutes. The length of time varies with the conditions: usually 2 to 5 minutes is long enough. Wash off the stain with water. The preparation is then ready to study with a microscope. If only a few moments' study is desired, the cover-glass can now be placed on a glass slide, in a drop of water, with the bacteria side down, and studied directly with the microscope. If, however, it is desired to preserve the specimen for future study, dry the cover-glass, then place a drop of Canada balsam on a glass slide, and upon this put the cover-glass, bacteria side down. Study the preparations thus made with a microscope. For this purpose it is necessary to have high magnifying powers. Because of the minuteness of the bacteria, practically nothing can be made out concerning their shapes, with a magnifying power less than about 1,000 diameters, and to obtain this it is common to use a $1/12$ -inch immersion lens. Place the prepared specimen on the microscope stage, and put a drop of immersion oil on top of the cover-glass. Lower the $1/12$ -inch lens into the immersion oil, and then carefully focus upon the bacteria. If the microscope has an Abbe condenser or a diaphragm below, it is best to have this widely open, for the colored bacteria are more easily seen with an open diaphragm. The bacteria are very small, and even with this magnifying power it may sometimes be difficult to make out their shape; but ordinarily their general appearance can easily be made out. Examine in this way all the cultures isolated in No. 14, and sketch.

***No. 16. Comparison of Yeast and Bacteria.** Rub up in a watch glass a bit of a cake of yeast with a little water. Place a drop of the liquid on a slide and examine directly with the microscope, using a $1/6$ -inch objective. Dry a little of the material on a slide and stain exactly as in staining bacteria (No. 15). Study with the immersion lens, comparing the yeast

cells as to *size* and *shape* with bacteria already studied. Hunt for cells showing *budding*. If these are not found they may be obtained in quantity by planting a little yeast in a weak solution of molasses in water and allowing to grow for a few hours in

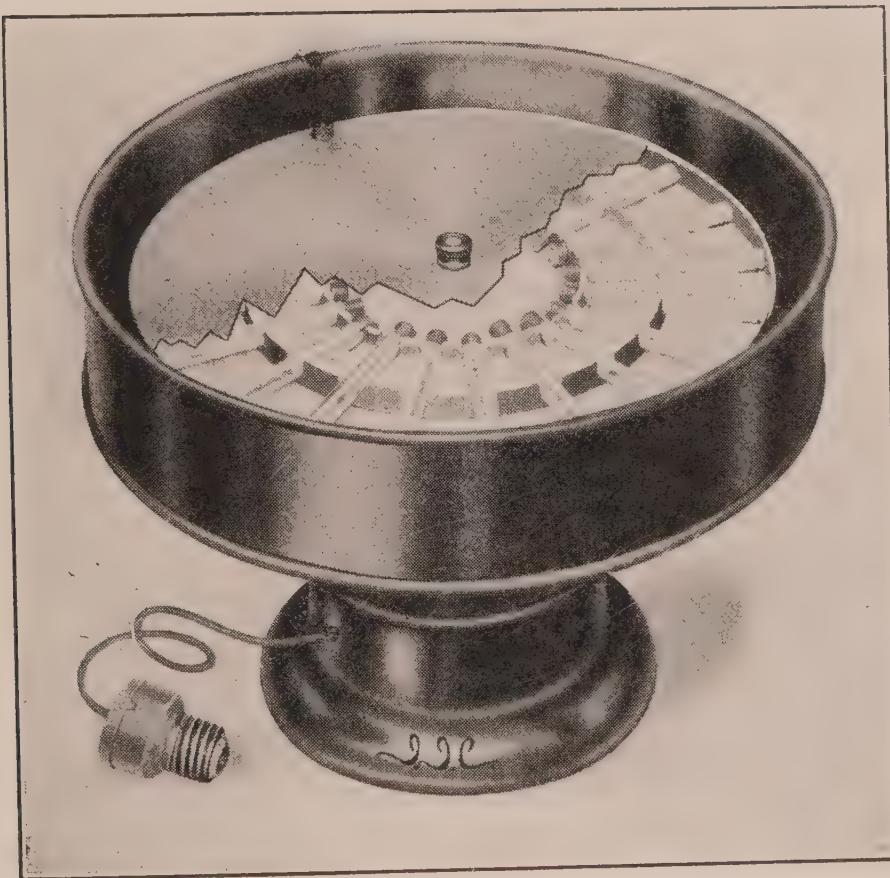


FIG. 81—CENTRIFUGE FOR MILK TESTING
(International Instrument Co.)

a warm place. Make a sketch of the yeast cells with buds, showing their relative size to that of bacteria.

No. 17. Direct Microscopic Study of Milk. For this a centrifuge is needed, having tubes with straight sides, and closed at the bottom with a rubber cork. (Fig. 81.) Place 2 c.c. of the samples of milk to be tested, after thorough shaking, in the tubes. It is necessary to fill enough tubes with milk from dif-

ferent samples, to balance the centrifugal machine on both sides. Rotate in the machine at a rate of 2,000 to 3,000 per minute, for 5 minutes. Remove the tubes from the machine and they will be found to have a layer of cream at one end, and a small slimy deposit at the other end next the cork. Holding the tube with the cream end down, remove the cream with a platinum loop, and pour out the milk gently. A slimy sediment will be left attached to the cork. Carefully remove the cork with its adhering sediment, and smear over the surface of a glass slide, with a drop of sterile water, to cover an area of exactly four square centimeters. Special slides marked with wax pencils into such areas are needed. This distribution of the sediment must be carefully done in order that it shall be uniform. After the thorough distribution, dry in gentle heat, or without heating, and stain by flooding the surface with dilute of methylene blue for a short time, and then wash the stain away. After again drying, the material may be mounted under cover-glass, or it may be examined immediately with an immersion lens without a cover-glass. There will be found on the slide a considerable number of stained bacteria, usually showing a variety of forms, and also a varying number of large cells, most of which have deeply stained nuclei, but unstained bodies. These latter are *leucocytes*, and represent cells of the character of the white blood cells from the cow, which have found their way into the milk. In some cases, leucocytes represent *pus* cells and are indicative of inflammatory changes in the animal's udder. Where the numbers are small, however, they give no such indication, for the normal milk of healthy udders will usually show considerable numbers of these leucocytes. To interpret the meaning of what is found under the microscope in these preparations, see page 284.

No. 18. Doane's Method of Determining Leucocytes. Place 10 c.c. of milk in the tubes of a centrifugal and rotate at about 2,000 per minute for four minutes. Carefully remove the cream

from the surface with a bit of cotton on the end of a rod, being careful not to leave any of the cream in the tube. By means of a small siphon remove the milk, keeping the tip of the tube just below the surface so as to avoid disturbing the sediment. Siphon away the milk to within about one-eighth of an inch of the sediment. Add two drops of a saturated alcoholic solution of *methylene blue* to the tube, and, after thoroughly mixing, place the tube in boiling water for two minutes to aid the staining. Add enough water to bring the total bulk either to one or two c.c., according to the amount of sediment. This will give a blue mass of stained sediments. To count their number an ordinary blood counter is used. This will have a counting chamber marked off into squares and holding exactly one-tenth of a cubic millimeter. Fill this counter with the stained sediment, cover with a cover-glass, allow to stand for about one minute for the leucocytes to settle, and then, placing under a microscope, count the number of leucocytes found in a single ruled square and calculate the numbers in the whole chamber. Remembering then that the chamber contains one-tenth of a cubic millimeter of the stained sediment, it is easy to calculate the number of leucocytes in the original milk. This should be calculated upon the basis of a certain number per c.c., and good milk should not have more than 500,000 per c.c.

No. 19. Staining of Tuberclle Bacilli. The staining of tubercle bacilli is rarely needed in dairying, but it is sometimes useful. The method is as follows. Make *Carbol Fuchsin* as follows:

Basic fuchsin	1 part.
Absolute alcohol	10 parts.
Carbolic acid (1-20 of water)	100 parts.

Prepare, also, a 20% solution of hydrochloric acid in water.

Spread the specimen to be stained (sputum or milk sediment from a centrifuge in the last experiment) upon a glass slide, and dry slowly in moderate heat. After drying, flood the slide

with the above stain, and allow it to stain for five minutes, warming slightly. Wash off the superfluous stain, and place in the acid solution, until all traces of red have been removed, which will require, usually, from about three to five minutes. Wash off the acid, dry and flood the slide for about half a minute with methylene blue solution. Wash again, dry and examine with a compound microscope, without a cover-glass. The tubercle bacilli will be found, by this method, to stain red, whereas other bacteria will be stained blue. It requires a little experience to be successful with this method of staining, but it is fairly easy after a little practice.

***No. 20. Qualitative Bacteriological Analysis.** This is very difficult and can be successfully undertaken only after considerable experience. A qualitative analysis of milk must begin with an estimation of the number of bacteria to be expected, in order that a proper dilution may be selected to give 200 to 1,000 colonies per plate. If the age and temperature of the milk are known, an approximate guess may be made. If the milk is less than 12 hours old and is kept moderately cool, a dilution of 100 and 1,000 may be chosen. If from 12 to 24 hours old, 1,000 to 10,000 dilution will be better. If the milk is kept as warm as 20°, higher dilutions are needed, sometimes as high as 1,000,000 in warm milk 24 to 36 hours old. If it is kept cool, 50°, but if 48 hours old, it will be likely to require 10,000 to 30,000. These numbers are, unfortunately, not very definite.

Secure some milk not more than 6 hours old. Dilute 1,000 times and make several litmus gelatin plates exactly as in experiment II.

It is always best to use two or three different dilutions in order, if possible, to insure proper results in one set, at least. At the end of two days examine the plates to see whether rapidly liquefying colonies are liable to spoil the plates. If liquefiers are abundant the plates must be studied at once; but if not, it is best to leave them for from 4 to 6 days before study.

When the plates are ready, count as follows:

- a. The number of liquefying colonies. These may roughly be separated into those that *liquefy rapidly* and those that *liquefy slowly*, giving two easily distinguishable types.
- b. Count the number of *Bact. lactis acidi* (see page 30).
- c. Count the number of *B. aerogenes* (including *B. coli*). These can be recognized by their brilliant acid, large surface colonies and frequent gas bubbles.
- d. Count the number of colonies that neither produce acid, nor liquefy gelatin, and group them together as *neutral species*. This last group will contain many different kinds of bacteria, and may, with experience, be differentiated into several other types; but it is hopeless for the beginner to do much more than is indicated above in qualitative analysis.

***No. 21. Separation of the Common Species of Bacteria from Milk.** Allow some milk to stand at about 70°. As soon as it begins to sour, but before it curdles, dilute 1 c.c. 500,000 times and make litmus gelatin plates. Allow to grow four days at 70° unless liquefiers make it impossible.

- a. Determine the total number of bacteria in 1 c.c. of milk.
- b. Determine the number of acid bacteria per c.c.
- c. Determine the number of liquefiers per c.c.
- d. Determine the *percentages* in each case.
- e. Isolate a colony of *Bact. lactis acidi*. This may be recognized as follows: It is an intensely acid colony, rather opaque, always below and never on the surface. It is small, only just visible to the naked eye and when examined under a low power microscope it frequently, though not always, shows a slight roughness, looking like minute spines around its edge. (Fig. 16.) With a platinum needle lift out one of these colonies and inoculate into a gelatin tube by stabbing the needle directly into the gelatin as shown in figure 82. After growth purify as described in experiment 13, label and set aside for future use.
- f. Look over the plates made in this and the last experiment

and see if any large (usually about the size of a pinhead) colonies appear on the surface, white in color and intensely acid. If so isolate one, preferably one that shows a gas bubble be-



FIG. 82—MAKING GELATIN STABS

neath or beside it. Inoculate upon an agar slant, labeling it *Bact. aerogenes*(?). Whether it is really that species will be determined later. If no gas bubbles appear, isolate several of

the large, acid, surface colonies and some of them will probably prove to be the species desired.

In the same way isolate and inoculate on agar slants one rapid and one slow liquefier, and several of the neutral type of colonies.

After about two days' growth purify the cultures and reserve for future study.

***No. 22. Milk Agar.** Add 1.5% agar to some skim milk. After half an hour boil (better to heat in an autoclav) until the milk curdles into a custard. Replace water of evaporation—adjust reaction to 1.5% acid—filter through absorbent cotton—place in tubes and sterilize in the usual way. After the third sterilization slant the tubes and allow to harden. This milk agar is not so transparent as ordinary agar, but the lactic bacteria grow upon it more readily. Inoculate the purified cultures obtained in No. 21 upon the milk agar slant, and reserve for future use.

No. 23. Oidium Lactis. Procure a little soft cheese from market, preferably of the Camembert or Brie type.¹ Pour out into a Petri dish a tube of plain gelatin, and into another a tube of litmus gelatin. Allow to harden. With a platinum needle scrape off a little of the growth on the rind of the cheese and touch it upon the surface of the gelatin at several spots. It is well to try several parts of the cheese rind in this way. Cover, and after two days' growth it will usually be possible to find spreading colonies of *Oidium* on the plates. They are thin, and spread rapidly over the surface; they may be recognized under the microscope because at first they are seen to be composed of threads like a mold, which soon break up into short sections. The colonies usually become about $\frac{1}{4}$ inch in diameter. (See Fig. 29.)

***No. 24. Cheese Mold.** On the same plates, after 2 or 3 days,

¹ If such soft cheese is not to be found conveniently, *Oidium lactis* can usually be found in samples of old tub butter.

will appear a white mold. This grows more slowly than the *Oidium*, and its threads do not break up into spores. Spores form on the surface upon special branches. (See Fig. 63.)

Both the *Oidium* and *mold* may be isolated for future study if time permits.

No. 25. Abnormal Fermentations. If any unusual types of fermentation can be found, like slimy milk, bitter milk, tainted milk, etc., they may be studied to advantage. Gelatin plates should be made from such milk and the different kinds of colonies isolated. After purifying, these should be inoculated into sterile milk to see if any of them produce the trouble seen in the original milk. If such is found it may sometimes be traced to its source by hunting for it in various places around the dairy; *e. g.*, in the milk cans—cows' teats—dairy water—hay dust, etc. No definite directions for this can be given, for each problem will need different treatment.

***No. 26. Potato Tubes.** Carefully wash and peel a large potato. With a special cutter (Fig. 83), or with a broken test tube with sharp edges, cut out some potato plugs. Cut each obliquely in two parts and soak in running water 12 hours. In the bottom of some large test tubes place a little cotton and enough water to cover it. Place a single potato slant in each tube (Fig. 83) and sterilize by one-half hour steaming on three successive days.

***No. 27. Fermentation Tubes.** To 200 c.c. of bouillon prepared as in (4) add 2 grams of *lactose* (milk sugar). To a second 200 c.c. add the same amount of *dextrose* (grape sugar), and to a third lot add *saccharose* (cane sugar). Dissolve by heating—filter through filter paper and pour into fermentation tubes (Fig. 20), completely filling the closed arm and about one-half the bulb. Sterilize as usual. If gas collects in the arm, remove by tilting the tube.

***No. 28. Milk Tubes.** Procure some fresh skimmed milk. Test the reaction with litmus paper and if acid add sufficient NaOH to bring to neutral point of litmus paper. Place in tubes, about

10 c.c in each. Sterilize by steaming one-half hour for three successive days.

No. 29. Testing Characters of Bacteria. Inoculate the several bacteria previously isolated and purified, into various media. In doing this it is best to make first a fresh agar slant from each

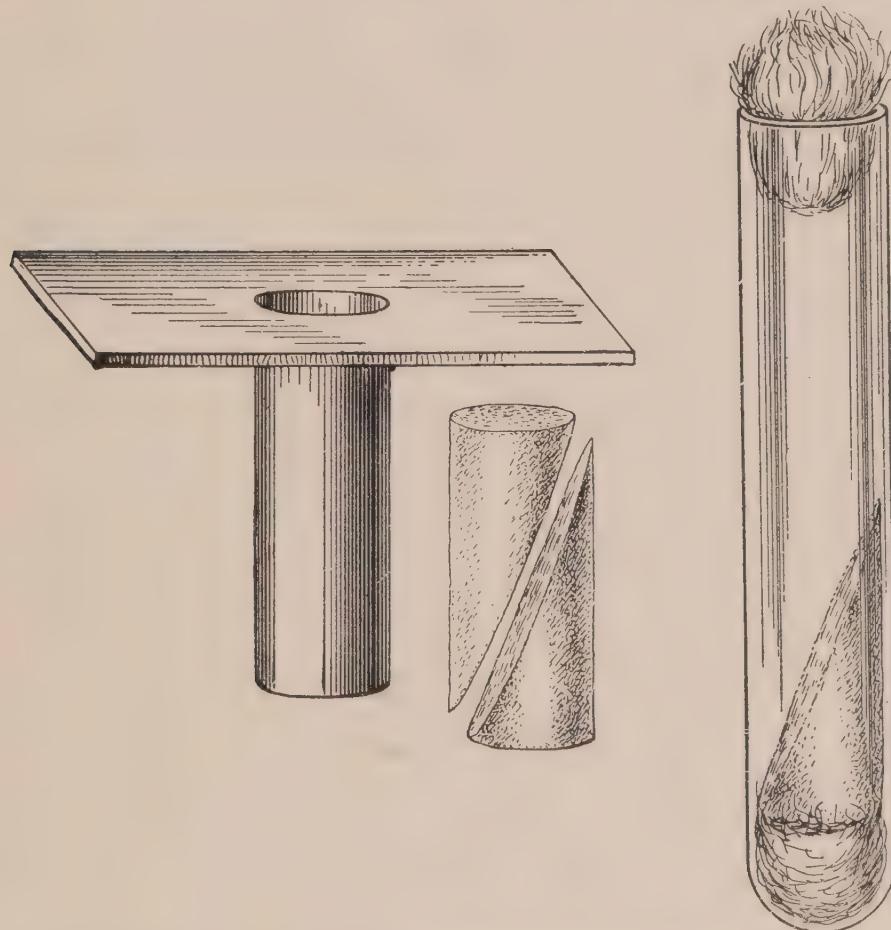


FIG. 83—CUTTER FOR MAKING POTATO PLUGS, METHOD OF CUTTING THEM AND PLACING THEM IN TUBES

bacterium to be tested, so that the inoculation may be made from a culture not over 24 hours old. Inoculate with a small amount of the growth from the agar surface, the following:

a. An agar slant. *b.* A gelatin stab. *c.* A tube of plain bouillon. *d.* A dextrose fermentation tube. *e.* A lactose fer-

mentation tube. *f.* Two milk tubes, one to be kept at 70° and the other at 98° . *g.* Two potato tubes, one at 70° and one at 98° . Allow the cultures to grow several days, examining each

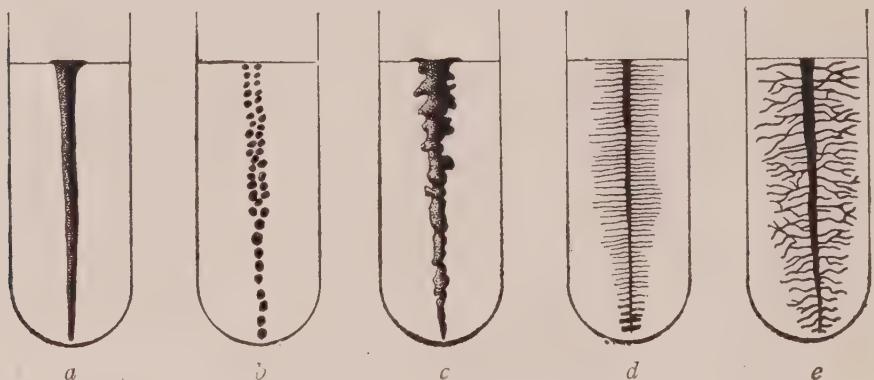


FIG. 84—TYPES OF GELATIN STAB GROWTHS

a. Filiform. *b.* Beaded. *c.* Echinate. *d.* Villous. *e.* Arborescent

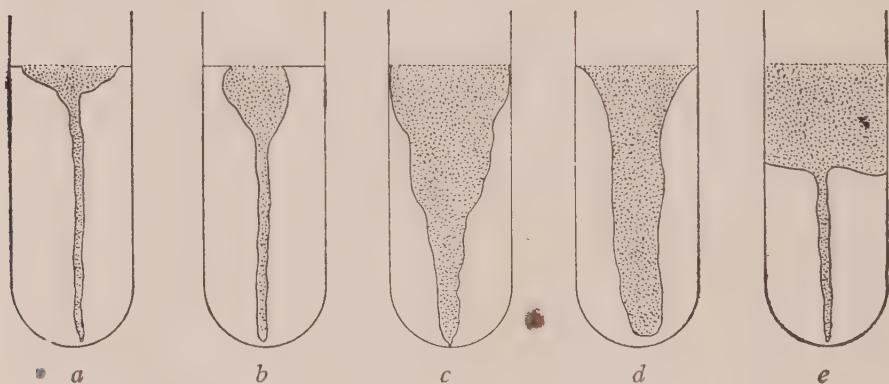


FIG. 85—TYPES OF GELATIN LIQUEFACTION

a. Crateriform. *b.* Napiform. *c.* Infundibuliform. *d.* Saccate. *e.* Stratiform

day. For each species of bacterium make a careful record, noting especially the following points:

Agar slant. Note the type of surface growth as compared with figure 19.

Morphology. Stain and study with the microscope, noting shape—formation of chains—spores—determine motility. (See No. 31.)

Gelatin stab. Note liquefaction—needle growth—surface

growth—color. Compare the growth with the different types shown in figures 84 and 85, and determine with which each agrees. Note the amount of elevation of the surface growth and compare with figure 86.

Bouillon. Note turbidity—scum—sediment.

Fermentation tubes. See below (No. 30).

Milk tubes. Note at 70° and 98° the development of acid as shown to litmus paper—curdling—separation of whey—appearance of *gas bubbles*—subsequent *softening of the curd*, called digestion.

Potato tubes. At 70° and 98° . Note *color* of growth—*abundance* of growth—*texture* of growth—*discoloration* of potato.

By such characters as above determined, different kinds of bacteria are distinguished and described. For a complete description more characters than those mentioned are necessary. The characters of the cultures which have been named, *Bact. lactis acidi* and *Bact. aerogenes*, should be compared with those given on page 30 to see if they agree with the characters there given.

***No. 30. Fermentation Tubes.** Inoculate a series of fermentation tubes (dextrose, lactose and saccharose) with different bacteria. Among those chosen should be one culture of *Bact. lactis acidi*, and one of *Bact. aerogenes*. In two days, note whether gas has been produced in the closed arms, and, by means of litmus paper, whether the bouillon has become acid. If gas shows in the fermentation tubes, determine the gas ratio as follows:

Without disturbing the gas, fill up the bulb to the top with a 2% NaOH. (By a mark, note the level of the gas in the glass arm.) Place the thumb over the opening in such a way that there will be no air bubble between the thumb and the surface of the liquid. Now, invert the tube, allowing the gas to flow out into the bulb, and, by turning back and forth, mix the gas with the NaOH solution in the bulb, keeping the thumb very

tightly closed over the opening. After thoroughly mixing, turn the tube once more so that all of the gas will be in the closed arm. Remove the thumb; it will usually be found that the level of the gas in the closed arm rises. If so, it will be due to the fact that CO_2 has been dissolved by the NaOH solution. By determining the amount of the gas before and after the test, the proportion of CO_2 to the gases not thus dissolved may be obtained. This is called the *gas ratio*. Some gas-producing species of bacteria produce large quantities of carbon dioxide, and small quantities of other gases, while other species produce no carbon dioxide.

From one or two of the test tubes inoculated above, that show an acidity, determine the amount of acid produced. For this purpose remove from the tube either 5 or 10 c.c. of the liquid, dilute it with 10 times its bulk of water, heat and titrate, as described in experiment No. 4, *b*.

No. 31. To Determine Motility. Inoculate a little of the bacteria growth of No. 21 into a test tube of bouillon. Allow it to



FIG. 86—TYPES OF SURFACE ELEVATION

1. Flat. 2. Raised. 3. Convex. 4. Pulvinate. 5. Capitate. 6. Umbilicate.
7. Umbonate

grow for about 24 hours. By this time the liquid will become somewhat cloudy. Transfer a loopful of this material to a glass slide and place upon it a cover-glass. Place the whole under microscope, preferably using a 1/6-inch objective, and narrow the diaphragm below the stage until most of the light has been cut off. Now focus very carefully upon the bacteria under the cover-glass, and, if they are motile, it will be seen that they are moving around through the microscope field with more or less rapidity. If they are stationary, it may be assumed that they have no flagilla. This test requires great care, for

with some slowly moving forms it is not always easy to detect the motion with certainty; but it can easily be done with a little study.

No. 32. Germicidal Action of Milk. Procure some milk fresh from the cow; dilute 1 c.c. of this milk 100 times and make 6 agar (or gelatin) plates. Cool the rest of the milk at once to 70°. At the end of 2 hours make 6 more plates in exactly the same way as above. In 2 hours more make 6 more plates. After proper time for growth, count the colonies and determine whether there has been a decrease in numbers of bacteria in the first few hours after milking.

No. 33. Aseptic Milk. Sterilize a liter flask plugged with cotton. Wash the hands thoroughly in hot water and soap, and put on a clean milking suit. Remove a clean cow from the barn into the open air, brush the dirt from the flanks and udder with a clean brush and then with a damp cloth. Wash the udder and especially the teats with a 10% borax solution. After drawing about a dozen jets on to the ground, remove the plug from the flask and, placing its mouth very close to the teat, milk it about half full, and replace the cotton plug. Carry at once to the laboratory and make a litmus gelatin plate immediately, diluting 10 times. After 2 or 3 days' growth, count the colonies. Are there any acid colonies of the *Bact. lactis acidi* present? If time permits, isolate the colonies; purify and determine the general class of bacteria.

Allow the rest of the milk to stand at about 70° to see if it will sour normally.

No. 34. Effect of Temperature on Species of Bacteria in Milk. Procure some fresh milk and make several litmus gelatin plates, diluting 100 times. Divide the milk in three lots, placing one at about 50°, one at 70°, and one at 98°. At the end of 24 hours make plates from each, diluting that kept at 50° 2,000 times, that at 70° 10,000 and 100,000 times, and that kept at 98° 500,000 times. After another 24 hours, make some plates from

the sample at 70° , diluted 10,000 times. Allow all plates to grow at room temperature until the colonies are well developed. Determine the total number of bacteria in each sample, and the number and percentage of each kind of bacteria that can be clearly distinguished by its colony. Compare the numbers and percentages of the different species at the different temperatures.

***No. 35. Bacteria of the Air.** Place one dozen tubes of sterilized milk—with cotton plugs removed—in various places around the barn, dairy, house and laboratory. Leave undisturbed for 6 hours. Then replace the plugs, carry all to laboratory, and place at about 20° . Watch for several days and notice whether all appear to undergo the same kind of fermentation. Do any of them sour normally? Do any remain unchanged?

No. 36. Types of Bacteria from the Air. After the tubes in No. 35 show signs of fermentation make a gelatin plate from each, using a loopful of the milk in a water blank and inoculating gelatin tubes with a small loopful of the water blank dilution. After proper growth examine the colonies in the different plates and compare with each other. What does the experiment teach? If time permits, the colonies may be isolated, purified and further studied, as in No. 28.

***No. 37. Dust Plates.** Pour 12 tubes of melted gelatin into Petri dishes; cover and allow to harden.

a. During the milking of a cow hold one of the above gelatin plates under the udder of the cow, close to the milk pail; remove the cover so as to allow any dust particles to fall on the gelatin, for half a minute. Replace the cover and carry to the laboratory.

b. Place 3 of the flasks at different places in a cow barn several hours after feeding. Remove the covers for 3 minutes. Replace covers and carry to laboratory.

c. Repeat *b* ten minutes after feeding the cows with hay.

d. Repeat *c* after feeding with other foods.

e. Expose one plate the same length of time out of doors.

After the above plates have developed count the colonies on all the plates and compare.

No. 38. Bacteria in Hay. Soak a little hay in warm (not hot) water for an hour. Shake well and inoculate three litmus gelatin tubes, the first with one, the second with two and the third with three loops full of the hay infusion. Pour into Petri dishes, incubate and study as usual. Compare the numbers of liquefiers and non-liquefiers. Are there any acid forming colonies?

No. 39. Bacteria in Grain. Repeat the above experiment, using some grain feed instead of hay.

***No. 40. Value of Cleaning Milk Bottles.** Procure 4 glass bottles in which milk has been standing for a day; ordinary unwashed milk bottles are best.

- a. Wash one with cold water.
- b. Wash one with hot water and soap.
- c. Wash the third and sterilize in steam.
- d. Leave fourth unwashed.

Fill all four with fresh milk. Place all side by side at a room temperature and notice the time of souring in each case.

***No. 41. Bacteria in the Milk Pail.** Place 100 c.c. of sterile water in a milk pail that has received a simple washing. With a little absorbent cotton rub this water around thoroughly; allow to stand a few moments, and, after another washing around the pail, remove 1 c.c. of this water and plate in agar. Count the colonies.

- a. Repeat, using (1) a dirty pail; (2) a pail simply steamed for a few seconds in a steam jet, and (3) a thoroughly sterilized pail.

No. 42. Bacteria in Manure. With a sterilized platinum loop transfer a small bit of either fresh or dried manure from a cow to a sterile water flask. Mix thoroughly. Transfer two loopfuls of the mixture to a tube of melted agar. Pour into Petri dishes and after growth count the number of colonies. To determine

the *actual number* in manure it is necessary to weigh the original bit of manure and make subsequent calculations.

No. 43. Isolation of B. Coli. Repeat No. 42, using litmus agar. If any strong acid surface colonies appear, choose one that shows a gas bubble if possible; isolate, inoculate on an agar slant, and purify as usual. From the purified culture inoculate a glucose fermentation tube. If gas appears, examine the culture and determine whether the bacterium is a short motile rod. If so, the species is probably *B. coli*; if it is not motile, it is probably *B. aerogenes*. Compare with page 34.

No. 44. Bacteria on Hair. Melt a tube of agar and one of gelatin, and pour into Petri dishes. After they have hardened place upon the surface of each one or two hairs from the flank of a cow. Two or three days later examine, and note the numerous bacterial colonies growing along the course of the hairs.

***No. 45. Fore Milk.** Draw the first 3 sets of milk from one teat of a cow into a sterile test tube. Now milk the cow about one-half dry and draw 6 more jets in a second sterile tube. Remove to laboratory and plate at once in plain gelatin, diluting 10 times. Compare the numbers and kinds of colonies in the two sets of plates.

No. 46. Bacteria on the Hands. Wash the hands thoroughly in 200 c.c of sterile water. Place 1 c.c. of this water in a tube of melted agar. Count the colonies and calculate the number of bacteria removed from the hands by the washing.

No. 47. Estimation of Dirt in Milk.¹ Procure some milk from a clean and carefully kept dairy and also some from a dairy where the milking is done in a careless, slovenly fashion. Determine the amount of dirt in the milk in each case, as follows:

a. First method. Place a quart or a liter of each sample of milk in a tall glass cylinder, and allow it to stand for several

¹ This experiment is impossible without a chemist's balance and apparatus for drying, such as used in quantitative chemistry and analysis.

hours. A deposit of dirt will collect at the bottom, which may easily be seen, by looking through the glass. With a siphon carefully remove the milk to within $\frac{1}{4}$ inch of the bottom, but do not disturb the sediment. Fill up with clean water and allow to settle again. Once more, after settling, siphon off the liquid, and fill the glass with water. Repeat this operation several times, until the water becomes fairly clear. Then, after allowing the material to settle again, siphon off a considerable portion of the liquid, and after thoroughly mixing the sediment with the remainder of the water in the cylinder, pour it through carefully weighed filter paper; wash the filtrate by means of water through the filter paper. Remove the filter paper and dry, until it comes to a constant weight. Weigh. The difference in weight between this and the original weight of the filter paper will give the weighed amount of dirt collected.

b. Second method. Place a definite quantity of milk, usually 10 c.c., in one of the glass tubes of a centrifugal machine. Set the machine in motion and rotate rapidly for ten minutes. By this time the sediment will mostly be thrown to the smaller end of the tube. The cream and milk are then to be carefully removed from the sediment with a pipette, and the tube filled up again with clean water and centrifugalized again. This is to be repeated until the water becomes clear, after which the sediment in the bottom of the tubes is to be carefully washed out with clean water upon a filter, and the rest of the procedure is as above.

c. Third method. Procure some bolting cloth of the *finest* mesh. The meshes should be fine enough to retain all the visible particles, but to allow fat particles to pass through. Place in a ribbed funnel, moisten with water, and pour through it one quart (or liter) of milk. Wash the material left on the cloth with water and then, with a mixture of ether and alcohol, equal parts. Wash the dirt on to a carefully weighed filter paper. Dry thoroughly and weigh as above.

***No. 48. The Covered Milk Pail.** Milk two cows, one into an ordinary milk pail with a flaring, open mouth and the other into a covered milk pail. Determine the amount of dirt in the milk in each case. Immediately after the milking, in each case, make a quantitative count of the number of bacteria, comparing the results together, and determine the value of a covered milk pail. The next day repeat the experiment, reversing the two cows.

***No. 49. Advantage of Immediate Cooling.** Immediately after milking, cool, by aerating, part of the milk, and place in sterilized jar. Fill another similar jar with milk not cooled. Fill a third jar with milk cooled by immersing at once in iced water. Place all three jars side by side in an ordinary room and compare the time of souring and curdling in the three specimens.

Cool night's milk immediately, as cool as possible. The next morning fill two jars, one with the night's milk that has been cooled and the other with the milk warm from the cow. Place both at a temperature of an ordinary room and compare the time of souring and curdling in the two cases.

No. 50. The Effect of Careful Milking. Compare the effect of milking, for six successive days, with and without precautions. The first three days use open milk pails and make no special attempt at cleanliness. The second three days use closed milk pails, groom the cows, wash the udders and perform the milking out-of-doors instead of in the closed barn. In each case make a quantitative analysis of the number of bacteria in the milk and compare the results.

***No. 51. Sterilization by Boiling.** Make a set of bacterial plates from a sample of milk in gelatin. Then boil the milk for five minutes in a flask closed with cotton and make another set of plates, diluting by 10. Allow the boiled milk to stand for 24 hours and make another set of bacterial plates, diluting by 100. Allow the rest of the boiled milk to stand in the laboratory until it shows signs of fermentation. Determine whether it has

soured, and describe any other notable change. After the plates have had an opportunity to grow, count the number of bacteria.

No. 52. Sterilization by Autoclav. Sterilize milk in a plugged milk flask for 15 minutes in the autoclav at a pressure of 15 pounds. Allow to stand for several days and then make gelatin plates from it and determine whether the milk was completely sterilized or not.

***No. 53. Pasteurizing Milk.** Obtain some milk that is from 12 to 20 hours old. Make a set of plates on agar, diluting by 1,000. Divide the milk into two lots. Heat one lot to a temperature of 140° for one-half hour, stirring frequently. Heat the other lot to 160° for 10 minutes. Allow both to cool and make agar plates from each, diluting by 100. After plates have grown, calculate the number of bacteria before and after pasteurizing at the different temperatures.

a. Repeat the last experiment, making plates, however, in litmus gelatin and calculating the number and percentage of acid colonies in the milk before and after pasteurizing.

No. 54. Pasteurizing Machines. If a pasteurizing machine is available, test its efficiency by several experiments. In each case a thoroughly mixed sample of milk should be first carefully tested by gelatin plates, and corresponding sets of plates should be made from the milk after pasteurization. Use the machine under varying conditions of temperature and time of pasteurizing, and determine the efficiency under the different conditions.

No. 55. Condensed Milk. Open a can of condensed milk and remove with a sterile platinum loop, or a sterile knife, a quantity of the condensed milk and dilute with sterile water. Make a series of bacterial plates with a loopful of the diluted milk and determine whether the condensed milk was sterile. If colonies develop in the plates, examine with the microscope and determine whether they are yeasts or bacteria.

***No. 56. Quantitative Analysis of Miscellaneous Milk Samples.** Obtain milk samples from several milkmen, learning, so far as possible, the age of each sample of milk. These should be collected in sterile bottles and kept on ice until they can be experimented with in the laboratory. Dilute each sample of milk 1,000 times and make agar plates from the final dilution; incubate at 98°. After 24 hours count the number of bacteria in each plate and compare the samples of milk.

No. 57. Qualitative Analysis of Miscellaneous Milk Samples. With the specimens above collected it will be useful to attempt a qualitative analysis, though this will be more difficult. The dilution of the milk must be varied according to its age and temperature. If it is fairly fresh—only a few hours old—a dilution of a thousand times is satisfactory. If it is 12 hours old, a dilution should be, at least, 10,000; and if 24 hours old, it should be as high as 100,000, or higher. The amount of dilution necessary may be determined by a direct microscopic study. Make and stain a slide from each sample of milk, as directed in No. 17. Count the number of bacteria per field. If the average number per field is less than 10, dilute the milk 100 times. If it is as high as 100, dilute 1,000 times; and if still higher, dilute in the same proportion.

After diluting the milk make six litmus gelatin plates from each sample. It is best to have three of these plates diluted twice as highly as the other three. After cooling, place at a temperature of 70°. After two days, examine and determine whether there are any rapidly liquefying colonies that are likely to destroy the plates; if so, make a study of the plates at once. If possible, however, keep the plates for four days before studying them.

Study of plates.—After the plates have grown (2 to 4 days) study as follows:

a. Determine *total number* of bacteria per c.c. of milk. Compare with the total number found on the agar plates.

- b. Determine the number of *liquefying colonies*.
- c. Determine the number of *acid bacteria* per c.c. Are they of the *Bact. lactis acidi* type?
- d. Calculate the *percentage of acid bacteria*—liquefiers and miscellaneous bacteria in the milk. By reference to page 181 draw a conclusion as to which samples were probably badly contaminated.

***No. 58. Bacteria in Fresh Cream.** Dilute one c.c. of freshly separated cream with sterile water. If this is separated by a separator, the dilution should be about 1,000. If the cream has been separated by the gravity method, the dilution should be higher, since the cream is older, and should be as high as 100,000. After diluting, make a series of four plates in litmus gelatin, incubate at 70° and study as described in No. 56.

***No. 59. Bacteria in Ripened Cream.** Repeat experiment No. 57, using, however, some ripened cream that is just ready for churning. In this case the dilution must be much higher, and probably never less than 1,000,000. After incubating determine the variety and numbers of bacteria present.

No. 60. Analysis of Cream at Successive Intervals. Obtain some sweet separator cream, and set it to ripen in the ordinary way. Make a series of litmus gelatin plates from the cream, when first separated, and others at 6 hours later, 12 hours later and at the time of churning. The dilution of cream in the successive experiments should be increased from about 1,000 to 1,000,000. Incubate at 70° and carefully determine the number of bacteria, their types and percentages. Note particularly the increase in the percentage of lactic acid bacteria at the successive periods of the ripening.

No. 61. Analysis of Butter Milk. Make an analysis of the bacteria in butter milk in litmus gelatin. The dilution in this case should not be less than 1,00,000.

No. 62. Analysis of Butter. Weigh out upon accurate chemical scales 5 grams of freshly made butter. Place this in a sterile

mortar, with 9.5 c.c. of sterile water. Rub the water and the butter together thoroughly, so as to distribute the bacteria as uniformly as possible through the water. This mixing should be continued for some time, for, at best, many of the bacteria will remain clinging to the fat. Dilute this mixture 10,000 times and make a series of agar or litmus gelatin plates. Incubate and count as usual.

After 24 hours make a second series of plates from the same sample of butter, and repeat again in two days and one week. Obtain, if possible, a sample of butter several months old, and make similar analysis. In all cases determine the numbers, and, if possible, the proportion of acid bacteria and the liquefiers.

No. 63. Home Starters. Obtain some clean milk from a thoroughly healthy cow; place in sterile vessels, and cover to keep out the dust. Set aside at about 65° until the milk is soured but not quite curdled. Examine carefully by taste, by smell and by general appearance, to determine whether the curd seems to be of a type favorable for butter-making. It should be smooth in appearance, and have a clean, sharp taste and pleasant odor. Use this, if convenient, as a starter for ripening cream.

***No. 64. Making a Starter from a Commercial Culture.** Sterilize a quart of milk by boiling half an hour, or half an hour in an autoclav at 10 pounds pressure. After cooling to about 80°, pour into it the contents of a package of commercial butter starter, stir thoroughly, cover and allow to stand at 65° to 70° for 24 hours. The milk should by this time be sour and nearly ready to curdle, and may be used at once as a starter. If a larger amount is needed, pasteurize several gallons of cream by heating to 155° for one half hour. Cool to 80°, and pour into it the quart of starter prepared from the commercial culture. Allow to stand at 65°, after which it is ready for use.

No. 65. Bacteriological Analysis of a Commercial Culture. Make a bacteriological analysis of some commercial culture to determine whether it is pure or not. For this purpose put a

small quantity of the culture from the package into a water blank, and, by thorough agitation, distribute evenly through the water. From this inoculate litmus gelatin tubes, some with one, some with two and some with three loopfuls of the diluted culture. Pour into Petri dishes, incubate at 70° , and after three days examine to see whether any bacteria can be found in the plate except lactic germs.

No. 66. Effect Upon Butter of Pasteurizing Cream. Divide a lot of cream which is to be made into butter into two parts, pasteurize one at a temperature of 155° for 15 minutes, leaving the other without pasteurization. Add to each the same amount of starter. Ripen in the usual way, churn and make into butter, and compare the products, to see if any difference can be noted between the butter from pasteurized and non-pasteurized cream.

No. 67. The Effect of Different Bacteria on Butter. The following experiment can be performed only where conveniences are at hand for churning small quantities of cream. Pasteurize a considerable quantity of cream, as above described, and divide into several lots, placing each in a separate vessel. Inoculate each lot with a starter made from different species of bacteria, which have been isolated during the laboratory experiments. Allow the cream to ripen in the normal way for the usual length of time, and churn into butter. Compare the texture, taste and aroma of the butter obtained in the different experiments.

No. 68. The Effect of Light Upon Butter. Place two lots of butter, one in a bright light and one in the dark, and after several days compare them as to appearance, smell and taste.

No. 69. Ripening of Cream at Different Temperatures. Divide some cream into three lots. Place the usual amount of starter in the cream, and ripen one lot at 50° , one at 65° and one at 85° . After proper ripeness has been reached (determined by acid test), churn and compare the butter which is obtained, unsalted, to detect any difference in flavor and aroma.

***No. 70. Analysis of Bacteria in Cheese.** Determine the num-

ber of bacteria in cheese exactly as above described for butter (see experiment 62), making inoculation into litmus gelatin plates, and determine the varieties of bacteria present. Make a series of plates in this way from a freshly made cheese and another series from some cheese ripened and ready for market. Compare the numbers and kinds of bacteria.

No. 71. Determination of Bacteria in Rennet. Dilute a small quantity of the rennet in a water blank and inoculate litmus gelatin plates. Incubate as usual, and examine at the end of two days. This experiment may be performed by the use of agar incubated at 98°.

No. 72. The Effect of Gassy Organism on Cheese. Inoculate a small quantity of milk with a starter made from a gassy organism (see page 287). After a few hours add rennet to the milk till curdled, and proceed in the usual way to make the curd into two cheeses. Leave one cheese for a few days in a moderately warm room, and notice the swelling due to the formation of the gas bubbles. Place the second cheese in a cool room with a temperature below 60°, if possible. Compare the formation of the gas bubbles in the two cheeses.

No. 73. Isolation of Molds from the Cheese. Obtain from the market a Camembert or Brie cheese. Pour into a Petri dish some litmus gelatin and allow to harden. With a platinum needle scrape off a bit from the surface of the cheese where it shows signs of mold, and inoculate it upon the gelatin by touching the surface with the needle in several places. Cover the dish as usual, and place at 70° for incubation. At the end of two or three days colonies of molds will be found growing on the surface of the plate.

Proceed as above described to obtain the mold of the ripened Roquefort cheese. The method of this procedure is the same, except that for inoculating the plate, the platinum needle should be dipped into some of the blue spots, which show in the middle of the Roquefort cheese.

If time should permit, the molds may be further studied. Allow them to grow for several days, when they will show a change in color, due to the formation of spores. The spores may then be taken up on the tip of a platinum needle and planted on other gelatin or agar plates, or upon agar slants, for preservation. The different species of molds are distinguished from each other by their methods of forming spores and, also, by their color. This subject hardly belongs to this book, but any dairy student who is working upon the problems of soft cheese-making should study the molds sufficiently to become familiar with the white mold of the Camembert, the mold of the Roquefort, and the common blue molds, which are found almost everywhere, but are mischievous organisms in the dairy.

No. 74. Bacteria in Soft Cheeses. From either or both of the cheeses in the last two experiments make litmus gelatin plates. After proper growth, study and determine whether there are any bacteria present except lactic acid germs. In most good cheeses of this type the ripened cheese should contain only lactic acid bacteria.

***No. 75. Effect of Different Species of Bacteria on Milk.** Inoculate a series of sterile milk tubes with a large number of different kinds of bacteria. It is well for this purpose to use all of the species isolated during these experiments, and as many other kinds of bacteria as may be available. Set all of the tubes aside at 70° , and at intervals of 24 hours examine each to determine the effect upon the milk of the different species of bacteria. The acidity should be tested by removing a loopful and placing on litmus paper, and special attention should be given to curdling, separation of whey, appearance of gas bubbles, the digestion of the curd and the appearance of odors as a result of the action of the different bacteria. The larger the variety of the different cultures used, the better.

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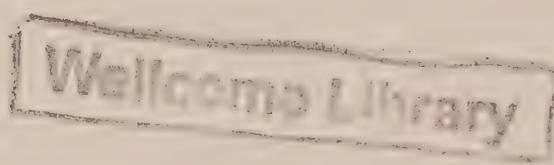
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